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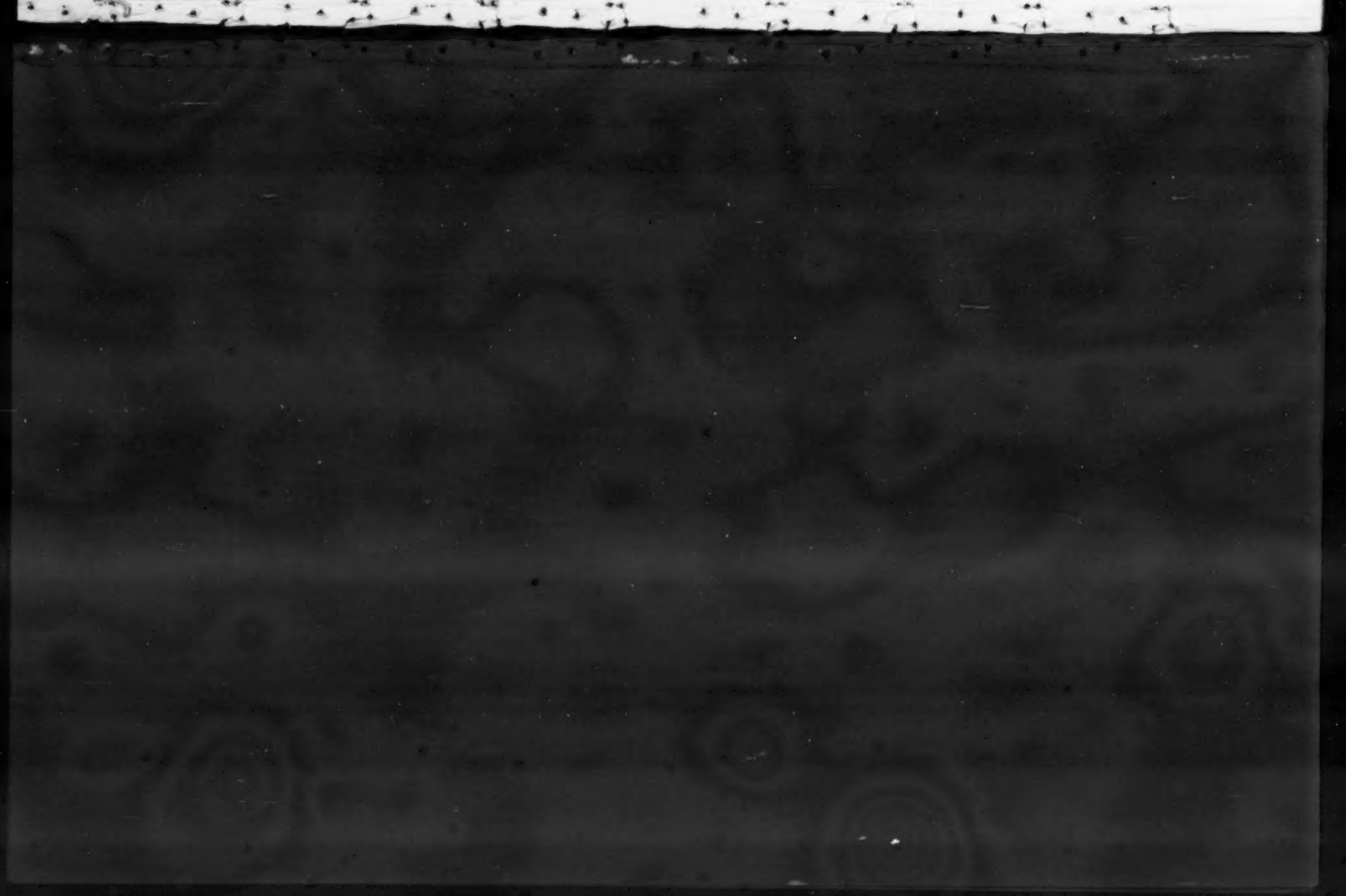

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EXPERIMENTAL POLIOMYELITIS IN MICE

OBSERVATIONS ON ITS GENESIS AND ON THE HISTOLOGIC CHANGES

WILLIAM EHRICH, M.D., AND CLAIRE FOSTER, B.S.

PHILADELPHIA

Previous experiments with the Lansing strain of poliomyelitis virus by Lillie and Armstrong [1] and with the S K New Haven fecal strain (J-S strain) by Jungeblut and Sanders [2] have shown that in mice these two strains produce a disease which closely resembles poliomyelitis in man and monkeys. Wolf [3] in comparing the histologic changes in the central nervous systems of these mice and of man, observed that the lesions were of essentially the same type and that there was constant involvement of the anterior horns of the cord. However, the mice infected with the J-S strain showed more severe involvement of the cortex, the rhinencephalon and, to a lesser degree, the cerebellum, while in the mice infected with the Lansing strain the pons and the medulla were more markedly affected. Moreover, 69 per cent of the mice inoculated with the Lansing strain became moribund or died within one week. Another 22 per cent died during the second week, and the remaining 2 animals, after twenty-three and ninety-five days. On the other hand, the J-S mice became ill and died within two to four days or rarely after five to seven days. Lillie and Armstrong, as well as Jungeblut and Sanders, expressed the belief that the lesions produced in mice were similar to those in man and monkeys both in topographic distribution and in individual type. On the other hand, Wolf, who studied the material of Jungeblut and Sanders, came to the conclusion that the pathologic changes did not in themselves substantiate this view, because the rather constant but mild anterior poliomyelitis was accompanied by severe encephalitis, as well as for other reasons. However, he admitted that these differences may be in part explained by the greater virulence of the J-S strain. Moreover, it should be noted that the mice of Lillie and Armstrong were uniformly inoculated intracerebrally, while in those of Wolf the injections were made variously: intracerebrally, intravenously, intraperitoneally, subcutaneously, intranasally and by gavage.

While investigating the relation of diet to resistance in a study of poliomyelitis we [4] had the opportunity to observe the natural history of this disease in three control series of mice which were inoculated with three different dilutions of the Lansing strain of the virus. We wish to report some of our observations, not only because they strengthen the view of Lillie and Armstrong and Jungeblut and Sanders, but because they throw new light on the genesis of this disease.

MATERIAL AND METHODS

The 145 mice used for this study were produced in the mouse colony of the Children's Hospital. Their ancestors were brought to this laboratory in 1934 from the colony of Dr. L. T. Webster and were sibling mated through eight generations. The last seven generations of this stock resulted from a close outcross technic which distributes and redistributes the genetic characteristics in a relatively small common pool.

The mice were maintained on the semipurified stock diet on which their ancestors had been reared for several generations.[4] Their average age was 27 days at the time of inoculation.

The stock virus suspension from which the dilutions were made was a 10 per cent saline suspension of mouse brain infected with the Lansing strain. The mice were divided into three groups. Each mouse after being anesthetized lightly with ether was inoculated intracerebrally with 0.03 cc. of one of three dilutions of the virus suspension; 42 mice (group 1) received a 10^{-1} dilution, 50 mice (group 2) a 10^{-2} dilution and 53 mice (group 3) a 10^{-3} dilution. Each animal was observed and weighed daily as long as it lived, and the clinical signs of infection in it were recorded as described in a recent publication.[5]

This investigation was aided by a grant from the National Foundation for Infantile Paralysis, Inc.

From the Philadelphia General Hospital, the Children's Hospital and the departments of pathology and pediatrics of the University of Pennsylvania School of Medicine.

1. Lillie, R. D., and Armstrong, C.: Pub. Health Rep. **55**:718, 1940.

2. Jungeblut, C. W., and Sanders, M.: J. Exper. Med. **72**:407, 1940.

3. Wolf, A.: J. Exper. Med. **76**:53, 1942.

4. Foster, C.; Jones, J. H.; Henle, W., and Dorfman, F.: J. Exper. Med. **79**:221, 1944.

5. Foster, C.; Jones, J. H.; Henle, W., and Dorfman, F.: J. Exper. Med. **80**:257, 1944.

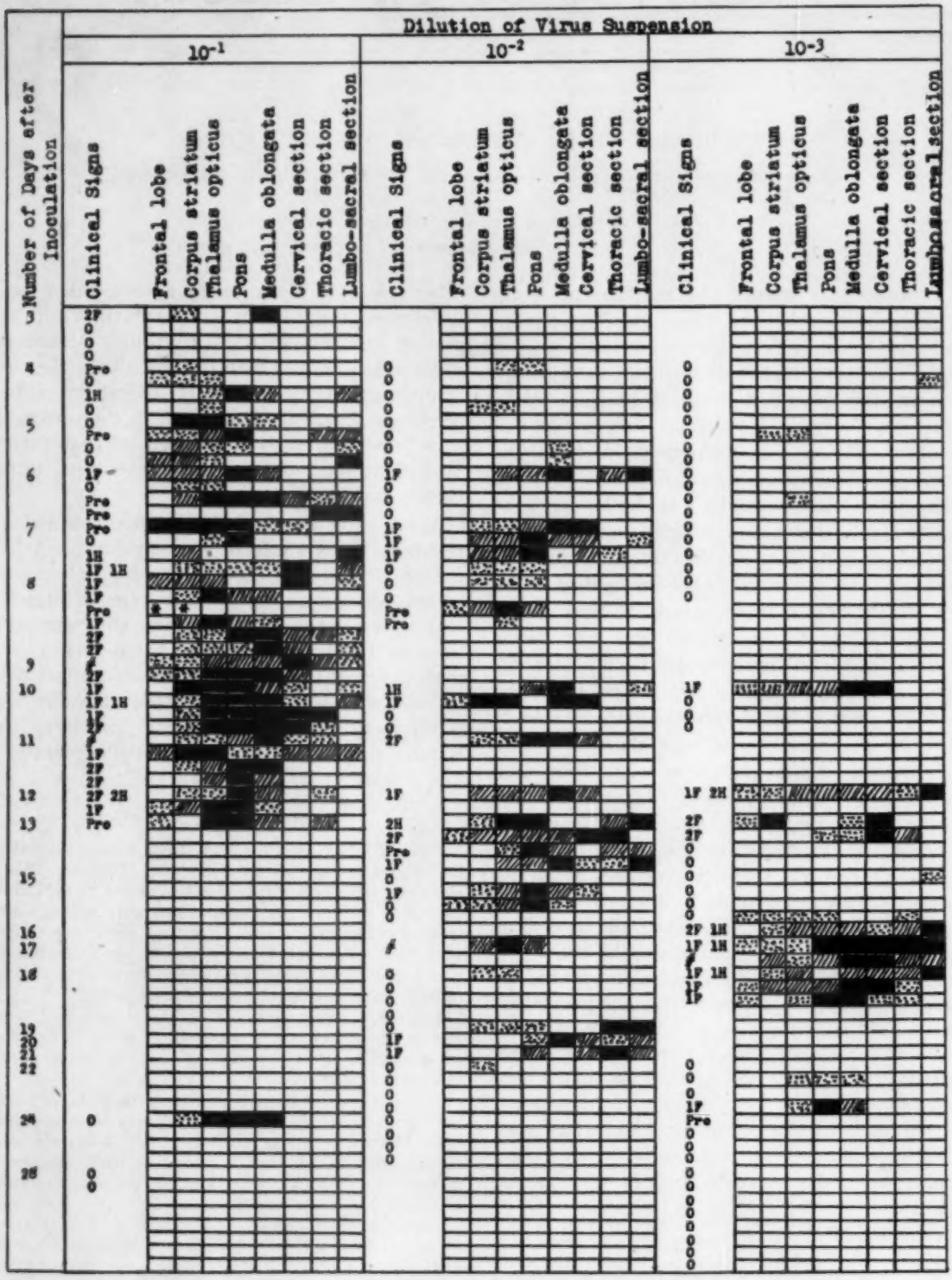

Chart 1.—Distribution of mild lesions (between 0 and + to between + and ++, dotted areas) and severe lesions (++ to +++, diagonally shaded areas), including the most intensive changes (solid black areas), in the central nervous system of mice inoculated with three different concentrations of virus, compared with the clinical signs noted. Under "Clinical Signs" *2 F* signifies that the two front legs were paralyzed; *1 H*, that one hindleg was paralyzed; *Pro*, that there were prodromal signs; #, that the mouse was found dead without clinical signs having been noted; **, that the lesion showed advanced autolysis.

Within the limits of the number of animals available, 4 mice were killed on the days specified in charts 1 and 2. Since it was desirable to fix the tissues immediately after death and at the same time to allow the affected mice to live until the disease was well developed, mice showing such signs of the disease as to suggest that they would not survive until the following day were killed first, and apparently normal mice, selected at random, were used to fill the daily quota.

The methods of dissection and the preparation of the tissues for microscopic examination have been described in an earlier paper.[6]

RESULTS

The histologic changes in the central nervous system of our mice closely resembled those described by Lillie and Armstrong,[1] Jungeblut and Sanders[2] and Wolf.[3] There were degeneration and necrosis of ganglial cells, infiltration of the gray matter with polymorphonuclear leukocytes, endothelial swelling and proliferation, perivascular infiltration with lymphocytes and other mononuclear cells and finally proliferation of fixed glial cells. Compound granular cells were not observed in our animals except in those parts of the brain where the virus had been injected.

The canal of injection was found in as many as two thirds of the mice studied: In 3 per cent of these animals it was located in the frontal lobes, and in 85 per cent, in the region of the corpora striata or the thalami optici or both; in the remaining 12 per cent the frontal lobes and the midbrain were both involved.

The sequence of pathologic changes began with granular and vacuolar degeneration and coagulation necrosis of ganglial cells and subsequent emigration of polymorphonuclear leukocytes. In an animal dead with the disease three days after inoculation, leukocytes were observed diffusely scattered throughout the medulla oblongata, and some had invaded necrotic ganglial cells. Swelling and proliferation of endothelial cells were also present, and there was already considerable perivascular infiltration with lymphocytes and other mononuclear elements in both the brain matter and the meninges. Glial proliferation became conspicuous only in those animals dying after four days, while both marked glial proliferation and marked perivascular infiltration were apparent only in animals which died after one week or more.

The sequence of events described here was best observed in the anterior horns of the cord, including the region of the internuncial neurons, but was apparent also in the medulla, in the pons and to a lesser extent in the brain stem from the corpora striata backward to the pons. The cortex and the cerebellum were rarely, and then only slightly, involved.

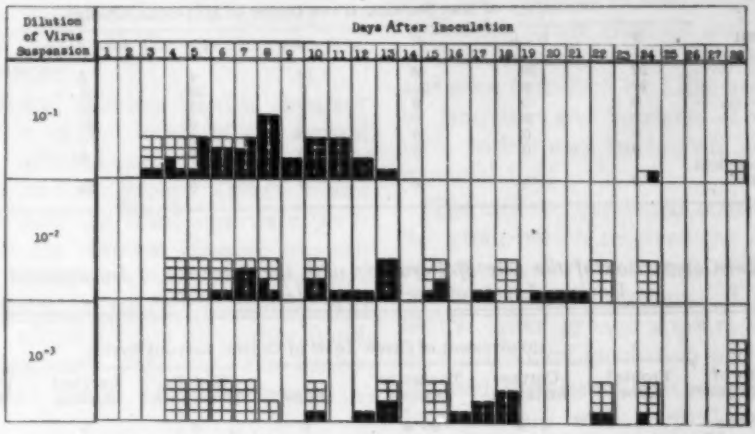

Chart 2.—Correlation of lesions in the central nervous system (right side of each column) with clinical signs noted (left side of each column). Each pair of squares represents 1 mouse. The white squares indicate no histologic changes (right side of column) or clinical signs (left side of column); the black squares, severe histologic changes (++ to +++) or prodromal signs, paralysis or spontaneous death.

As observed by previous investigators,[7] the distribution of the lesions was focal, much as in poliomyelitis in man and monkeys. The intensity of the lesions was greatest most frequently in the pons and the sections of the central nervous system adjacent to it; it was least in the thoracic portion of the cord (table 1). Our three series of mice seemed to differ only in that the animals receiving the strongest concentration of virus had the severest lesions in the thalamic region and the pons, while with the medium concentration the pons and the medulla, and with the lowest concentration the medulla

6. Foster, C., and Ehrich, W.: Arch. Path. **37**:264, 1944.

7. Lillie and Armstrong.[1] Jungeblut and Sanders.[2] Wolf.[3]

and the cervical swelling, were the most severely affected. This observation is in accord with the conjecture of Wolf that the greater involvement of the brain in the mice inoculated with the J-S strain was possibly due to greater virulence.

It is obvious from charts 1 and 2 that many animals receiving medium or small concentrations of virus never had the disease during the four week period of observation. It is also clear that the time of onset varied with the concentration of the virus: the greater the dose, the earlier the onset of paralysis.

In reading the paper of Lillie and Armstrong one gets the impression that all the animals received from it showed acute lesions, including necrosis of ganglial cells and infiltration with polymorphonuclear leukocytes. Moreover, these changes were severe in animals which became ill as long as three weeks after inoculation and in animals which died as early as the third day. Since the clinical intensity of the disease, too, was the same early and late after inoculation, our observation is interpreted to mean that the delay in time of onset of the disease when present was due to prolongation of the incubation period. It seems that only when the virus had multiplied and reached sufficient concentration to break down the resistance of the mouse did extensive tissue

TABLE 1.—*Comparison of the Degree of Histologic Change of the Central Nervous System with the Extent of Clinical Involvement*

Extent of Clinical Involvement	Percentage of Mice Showing Given Degree of Histologic Change							Animals
	0	0-+	+	+-++	++	++-+++	+++	
None	52	20	8	12	4	3	1	86
Prodromal signs	10	0	20	0	20	30	20	10
Paralysis of 1 limb	0	0	0	0	12	28	60	25
Paralysis of 2 limbs	0	0	0	0	6	25	69	16
Paralysis of 3 limbs	0	0	0	0	0	0	100	2
Paralysis of 4 limbs	0	0	0	0	0	0	100	1
Found dead without clinical signs having been noted	0	0	0	0	0	25	75	4

TABLE 2.—*Comparison of the Site of Paralysis with the Intensity of Involvement of the Various Levels of the Central Nervous System*

Animals	Site of Paralysis*	Involvement of Given Level of Central Nervous System							
		Frontal Lobe	Corpus Striatum	Thalamus Opticus	Pons	Medulla Oblongata	Cervical Section	Thoracic Section	Lumbosacral Section
Average of 85	None	0	0-+	0-+	0	0	0	0	0
Average of 33	1F or 2F	0-+	+-++	++	++	++	++	+	0-+
1	1H	0	0	+-++	+++	++	0	0	++
1	1H	0	++	0	0	0-+	0	0	++-+++
1	1H	0-+	0-+	....	++-+++	+++	....	0	+-++
1	2H	0-+	+-++	+++	+++	0	0	++	+++
1	1F 1H	0	+	+-++	....	+-++	+++	0	++
1	1F 1H	0-+	+	++-+++	++-+++	++-+++	+	0-+	++
1	1F 1H	+	+	+	++	....	++	++	++
1	1F 1H	0-+	+	++	0-+	++	++-+++	++-+++	+++
1	2F 1H	0-+	+	++-+++	++-+++	++-+++	+	++	+++
1	1F 2H	+	+	++	++	++-+++	++-+++	+-++	+++
1	2F 2H	0-+	+	+-++	+++	++-+++	0-+	+-++	0-+

* 1F or 2F signifies that one or two front legs were paralyzed; 1H and 2H, that one or two hindlegs were paralyzed.

acted alike; that during the first two and three days after inoculation necrosis of ganglial cells and infiltration with polymorphonuclear leukocytes were the prevailing features; that during the four to seven day period leukocytic infiltration was observed in only 1 of 8 animals and that vascular lesions and cellular gliosis were more prominent, and, finally, that after one week there were marked cellular gliosis, only inconstantly a few coagulated necrotic nerve cells and no polymorphonuclear leukocytes.

In our experiment with three different concentrations of the same strain of virus this course of events was not apparent except in some animals in which paralysis never developed. Almost all the animals which contracted the disease and change and paralysis develop. After this had happened, death ensued within one or two days.

The animals in which paralysis never developed or which were killed before the disease made its appearance showed only minor changes or no lesions at all. The pathologic changes when present first, included degeneration and necrosis of ganglial cells and infiltration with polymorphonuclear leukocytes, while later acute changes were absent and scar formation took their place. In these instances the immunologic and tissue reaction apparently was vigorous enough to combat the infection successfully.

If we compare now the pathologic changes in the central nervous systems of our mice with the clinical signs which were observed, it is clear

from charts 1 and 2 that prodromal signs, paralysis and spontaneous death were observed only in animals that showed severe lesions (graded 2 and 3 plus). Among all our animals we found only 3 presenting exceptions to this rule, namely, 3 mice showing prodromal signs but only mild or no histologic changes. The reverse was also true. Of all the animals showing severe lesions, only 7 had no prodromal signs or paralysis. Four of these animals were killed early after inoculation. It is conceivable that in these animals clinical signs were about to develop at the time when they were put to death.

The good correlation between lesions and clinical signs is apparent also from a comparison of the degrees of their intensity (table 1). In the absence of clinical findings only 8 per cent of the animals showed marked histologic changes. When paralysis was present, the greater the number of limbs paralyzed, the greater was the severity of the lesions.

A good reciprocal relation is also apparent from a comparison of the levels of the central nervous system affected and the locations of paralysis in our various groups (chart 1 and table 2). When only the front legs were paralyzed, the seat of the severest changes was not found to be the lumbosacral section of the cord except in 2 instances, in which the medulla was equally severely affected. On the other hand, when the hindlegs were paralyzed, the lumbosacral section was almost always severely involved.

These and the foregoing observations seem to strengthen the contention of Lillie and Armstrong and of Jungeblut and Sanders that experimental poliomyelitis in mice closely resembles the experimental disease in monkeys and the spontaneous disease in man. They also tend to show that the slight differences in distribution of lesions in mice inoculated with the Lansing strain and in mice inoculated with the J-S strain, which were observed by Wolf, may well be explained by differences in virulence of these strains.

SUMMARY

White mice were inoculated intracerebrally with the Lansing strain of poliomyelitis virus. Forty-two mice received 0.03 cc. of a 10^{-1} dilution of a 10 per cent saline suspension of mouse brain infected with the virus, 50 mice received 0.03 cc. of a 10^{-2} dilution and 53 mice 0.03 cc. of a 10^{-3} dilution.

Animals were killed whenever prodromal signs or paralysis made their appearance or at the intervals specified in charts 1 and 2.

The histologic changes and their distribution in these animals closely resembled those in rats and mice described by Lillie and Armstrong and by Jungeblut and Sanders. They also compared well with those in human and simian poliomyelitis.

The disease developed considerably earlier in the group which received the most concentrated suspension of virus than in those which received the lesser concentrations. The delay in onset in the latter groups appears to have been due to a prolonged incubation period.

Good correlation was seen between the histologic changes in the central nervous system and the clinical signs.

From the foregoing observations it is concluded that the mouse is well suited for the experimental study of poliomyelitis, particularly when large numbers of animals are required.

NONDEVELOPMENT OF THE SEPTUM TRANSVERSUM

WITH CONGENITAL ABSENCE OF THE ANTEROCENTRAL PORTION OF THE DIAPHRAGM AND OF THE SUSPENSORY LIGAMENT OF THE LIVER AND PRESENCE OF AN ELONGATED DUCTUS VENOSUS AND A PERICARDIOPERITONEAL FORAMEN

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It is generally agreed that the septum transversum in the human embryo gives rise to the central and anterior portion of the diaphragm, to the suspensory ligament of the liver and the ventrocaudal portion of the parietal pericardium.[1] Absence of these structures (presumably from failure of the development of the septum transversum) might therefore be expected to result in a complete pericardioperitoneal defect and in a displacement downward of the heavy and unsupported liver. This might also require a lengthening of the ductus venosus. A careful search of the literature reveals no instance in which any one of the foregoing defects has been reported. No biologic proof exists, therefore, that the septum has all these important functions. The observation of all of the aforementioned anomalies in a newborn infant with essentially normal diaphragmatic and pericardial development elsewhere prompts the publication of this report.

The mother was a 31 year old white primipara, and no history of another anomaly in the family and no untoward incidents occurring during the pregnancy were recorded. The infant was delivered at full term by podalic version and breech extraction following left occiput posterior presentation. The respiration of the infant was shallow and irregular, and he died three hours and forty-five minutes after delivery.

Autopsy.—The body was that of a white boy 4 hours old, weighing 3,500 Gm. and measuring 36.8 cm. in sitting height and 48.5 cm. in total length. There was considerable lividity over the right side of the face and on the body posteriorly, as well as much edema of the scrotum. A large herniation of the abdominal wall at the umbilicus measured 7 cm. in circular diameter and extended 6 cm. above the cutaneous surface. To it was attached a markedly edematous umbilical cord, which was smooth and shiny on the surface and measured 2.2 cm. in thickness.

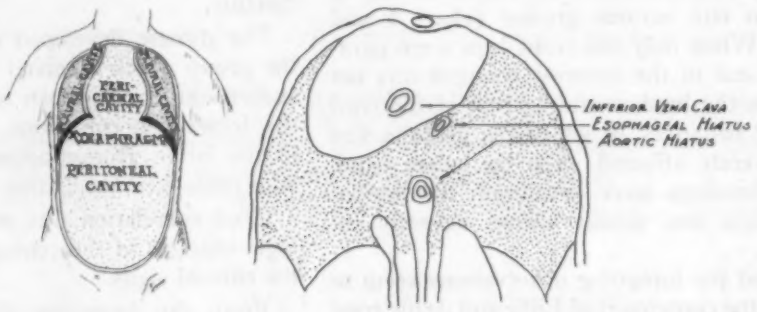

Fig. 1.—At left: Diagram showing the communication between the pericardial and the peritoneal cavity due to a congenital defect in the anterior portion of the diaphragm and in the pericardium. At right: Diagram of the diaphragm showing the defect in the anterior portion, including the section around the inferior vena cava. The rest of the diaphragm was apparently normal.

The entire liver was situated in the umbilical hernial sac. The stomach, the duodenum, the pancreas, the spleen and the intestine were in normal location and position except for downward displacement. The entire anterior portion of the diaphragm, to which the pericardium is ordinarily attached, was absent; the peritoneal cavity communicated directly with the pericardial cavity, which was greatly enlarged and distended (fig. 1). The defect in the diaphragm extended over to the right to include almost the entire right forward portion of the diaphragm and the section around the inferior vena cava. There was no defect in the esophageal hiatus. Neither the pericardial cavity

From the Department of Pathology, Baptist and Jefferson Hospitals.

Permission to publish the clinical data was given by Dr. James R. Garber.

1. (*a*) Bremer, J. L.: Arch. Path. **36**:539, 1943. (*b*) Southworth, H., and Stevenson, C. S.: Arch. Int. Med. **61**:223, 1938. (*c*) Jordan, H. E., and Kindred, J. E.: Textbook of Embryology, ed. 4, New York, D. Appleton-Century Company, Inc., 1942. (*d*) Arey, L. B.: Developmental Anatomy, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

nor the peritoneal cavity was continuous with either pleural cavity. The pleural cavities were fairly large and were not remarkable.

The inferior and the superior vena cava were normally formed and situated, but the heart was swung over so that the entire ventricular structure was situated on the right side of the vertebral column as well as to the right of the inferior and superior vena cavae (fig. 2). The pulmonary arteries were small; the pulmonary veins were fairly large. The ductus arteriosus was fairly large, and the foramen ovale had a well developed but somewhat fenestrated membrane situated in a deep

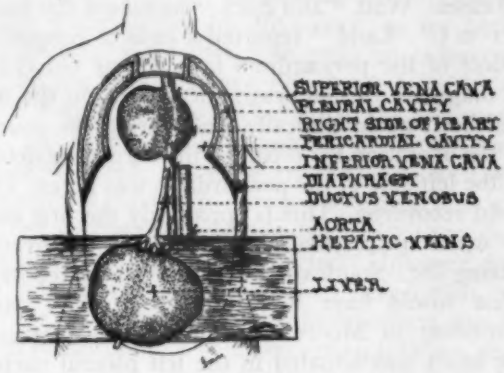

Fig. 2.—Diagram showing the downward displacement of the liver, which has dropped into an umbilical hernia, and the elongated ductus venosus.

sulcus. The right ventricle was approximately three times as large as the left ventricle, and the myocardium was proportionately thickened. The left atrium was also small, and neither atrium was enlarged. The right atrium had more the shape and the formation of an enlarged sinus venosus than a typical chamber. No defect in the interventricular septum or in the veins and arteries could be detected. No unusual muscle formation was present.

The lungs were partially aerated, the larger portions being somewhat rubbery and reddish brown. The spleen was enlarged, weighing 19.8 Gm.; it was well formed and in normal location. The liver was of a normal color and was not remarkable except for its position in the umbilical hernia. The pancreas was well formed and in normal position. The esophagus, the stomach and the small and large intestines were not remarkable. The appendix measured 4 cm. in length and 0.3 cm. proximally and distally. The large intestine was filled with dark green mucus. The stomach contained about 5 cc. of a straw-colored somewhat viscid fluid.

The adrenal glands weighed 11.8 Gm. The kidneys showed fetal lobulations. The ureters were not dilated. The urinary bladder was somewhat pointed toward the urachus but was otherwise muscular and contracted.

The prostate measured about 1.3 cm. in thickness. The right testis was in the scrotum, which was edematous. The left testis, the epididymis and the spermatic cord were situated in the left flank between the kidney and the brim of the pelvis.

The inferior vena cava was situated normally on the right half of the midline and showed no anomaly of position or structure. The hepatic veins emptied into a single vessel which extended 7 cm. in length from the herniated liver to the inferior vena cava. This single vessel was a continuation of the ductus venosus (fig. 3). The aorta was on the left and showed normal development of vessels and structures.

Authorization for an examination of the brain and the organs of the neck was not obtained.

The provisional anatomic diagnoses were: congenital defect of the diaphragm with communication between the peritoneal and the pericardial cavity; absence of the suspensory ligament of the liver; herniation of the liver into the umbilical sac; anomaly and elongation of the ductus venosus; rotation of the heart to the right; marked dilatation of the pericardial cavity; hypertrophy and dilatation of the right ventricle; dilatation of the sinus venosus; marked umbilical defect; marked edema of the umbilical cord and umbilicus; cryptorchism on the left; fetal atelectasis; edema of the scrotum; lividity of the face and of dependent surfaces.

Microscopic Examination.—The myocardium was infantile. The lungs showed considerable green pigment, and there were occasional hyaline cells in the alveoli. The alveoli of the lungs were only partially distended. The spleen was markedly congested, and there were considerable deposits of brown pigment in the sinusoid cells. The liver showed enlargement of the hepatocytes. The cytoplasm seemed granular and swollen. There was congestion of the blood vessels. There were rare hemopoietic foci and slight cellular infiltration of the portal spaces. Only certain portions of the liver showed the hydropic change in the hepatocytes. In the head of the pancreas the islets were few; islets in the tail were somewhat enlarged. There were large collections of lymphocytes in the interstices of several portions of the pancreas. The appendix had no abnormal neutrophils, lymphocytes or lipocytes. The mucosa of the small and the large intestine was well preserved. The adrenal glands had thick cortices, with some congestion. The kidneys contained primitive glomeruli and were congested. There were petechiae in the medullas. The testis and the epididymis were primitive. No hemorrhage was noted. The prostate was infantile. The lymph nodes had red blood cells in the sinusoids. There were no germinal centers in the lymph follicles. The adipose tissue was well developed, although individual lipocytes contained multiple vacuoles.

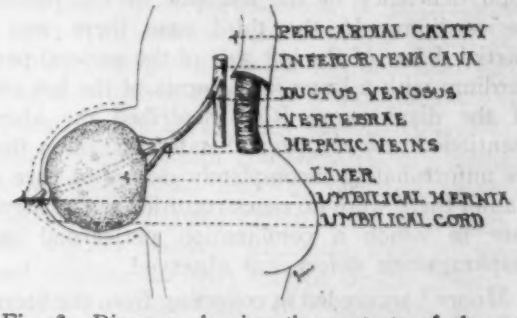

Fig. 3.—Diagram showing the contents of the umbilical hernia.

The red cells of the father and the mother showed the Rh factor; those of the infant did not.[2]

Additional histologic diagnoses were: slight aspiration pneumonia (early); focal storage of glycogen in the liver, slight hemopoietic foci in the liver.

2. Casey A. E., and Crowson, S. H.: Proc. Soc. Exper. Biol. & Med. **54**:320, 1943.

COMMENT

Defect of the pericardium is one of the rarer congenital anomalies. In 1559 Realdus Columbus [3] described a congenital deficiency of the pericardium observed in a young student of Rome. The authenticity of this observation has been doubted by subsequent writers, who believed that an adherent pericardium had been mistaken for absence of the membrane. In 1788 Baillie [4] gave a detailed description of a congenital defect of the pericardium, and the occurrence of this anomaly was established beyond doubt. In 1811 Otto [5] reported a case in which deficiency of the pericardium was associated with defects in the diaphragm in a 33 year old man. There was a wide defect on the left half of the diaphragm with a smaller one on the right. The pericardium was lacking except for two thin ridges ascending from the posterior mediastinum. In more recent times, Keith [6] reported a case in which there was, in addition to other malformations, a large defect of the left side of the pericardium; and in which the greater part of the left pleural cavity was occupied by the liver, the stomach and the spleen. Risel [7] described 3 cases in which pericardial deficiency was associated with diaphragmatic hernia, either true or false. In all 3 instances there were numerous other associated anomalies quite incompatible with life, and the infants were in reality monsters. In his first case there was a large true diaphragmatic hernia on the left associated with a large left parietal pericardial defect and a smaller right pericardial defect. In the second case there was a large false hernia of the left side of the diaphragm with almost total deficiency of the left side of the parietal pericardium. In the third case there was a partial defect of the left side of the parietal pericardium with a large false hernia of the left side of the diaphragm. Risel described the aforementioned case of Otto and stated that other than an unfortunately incompletely described case of Lambl there were no cases recorded in the literature in which a combination pericardial and diaphragmatic defect was observed.

Moore [8] succeeded in collecting from the literature 64 cases in which there was total or partial absence of the pericardium. Some were merely mentioned; others were described in detail. In all of the cases the defect was encountered at necropsy, the condition never having been diagnosed during life. Moore found 42 of these cases to be described in enough detail for analysis. His case was that of a dog in which the parietal pericardium was completely absent on the right; he considered it worthy of reporting, especially since the defect was a right-sided instead of a left-sided one as it usually is. Grant [9] added 1 case to the series and gained access to reports on 46 cases. Watt [10] and Beck [11] increased the number to 67. Ladd [12] reported a case of congenital defect of the pericardium in a 2 year old child. A diagnosis of diaphragmatic hernia on the left had been made clinically, and during the course of the operation for repair of the hernia the defect in the left side of the pericardium was noted. The child recovered. This is apparently the first case in which pericardial deficiency was discovered during life. Southworth and Stevenson reported what would have been the seventy-first case, according to Moore's count. In this instance, the heart was situated in the left pleural cavity, and there was not even a remnant of the left half of the pericardium. There was no diaphragmatic or peritoneal anomaly. They looked up all the references given by Moore and Grant, and after eliminating cases without protocols and those in which the anomaly was ectopia cordis or pericardial diverticulum, 54 cases remained, which they tabulated. A few cases of pericardial defect have been reported since that time.[13] In the case of Ronka and Tessmer [13c] the defect was recognized during life. It was in a 27 year old white soldier, who was admitted to the hospital because of a perforating gunshot wound. The absence of the pericardium was noted when an attempt was made to stimulate the heart manually, and the condition was confirmed at autopsy.

Thus it is found that the degree of deficiency of the pericardial sac varies from small circular defects to complete absence of the membrane. The most common type of pericardial defect is on the left with communication between the pericardial and the left pleural cavity. Occasionally the defect appears on the right,[14] and rarely on

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13. (*a*) Osgood, R., and Spector, B.: Am. J. Dis. Child. **61**:1028, 1941. (*b*) Kadin, M.: J. Michigan M. Soc. **38**:503, 1939. (*c*) Ronka, E. K. F., and Tessmer, C. F.: Am. J. Path. **20**:137, 1944.
14. Egbert, D. S., and Little, S.: Yale J. Biol. & Med. **8**:19, 1935. Moore.[8]

both the right and the left side.[7] It is generally agreed that the anomaly represents a patent pleuropericardial membrane resulting from a disturbance in the development of the celomic cavities and the pleuropericardial membrane.

In 5 cases there was associated with the pericardial defect a true or a false hernia of the left side of the diaphragm, and in 1 case there was associated with the defect a large left and a small right diaphragmatic hernia.

The first case of congenital diaphragmatic hernia was reported by Riverius[15] as far back as 1698, and since that time quite an extensive amount has been written on the subject. In 1912 Giffin[16] made a thorough review of the literature and found about 650 cases of diaphragmatic hernia reported, including traumatic as well as congenital hernia. In quoting the statement of another author, he said that herniation into the pericardial sac had been reported. However, he was unable to find the original description of the case, so he did not state whether there was a direct or an indirect communication between the pericardial and the peritoneal cavity. Hedbloom[17] analyzed 378 cases in which diaphragmatic hernia was surgically treated. In a table listing the sites of the hernias in 163 patients operated on, he listed one as pericardial. He neither described the case nor gave the exact reference. In 1931 he[18] collected 1,003 cases reported in the literature since 1900, in about one third of which the anomaly was classed as congenital. He noted that the congenital hernia typically involved the posterior portion of the diaphragm. In 1934 he[19] was able to collect 821 cases of nontraumatic hernia. He did not mention herniation into the pericardium and stated that, of the three large openings in the diaphragm (the esophageal, the aortic and the caval), the esophageal is the only one through which hernia occurs. Because at that time the validity of the technic of roentgen diagnosis was disputed by some observers, he excluded a large series of cases in which hernia of the esophageal hiatus was diagnosed chiefly roentgenologically but in which the diagnosis was not confirmed by operation or autopsy. After marked advances were made in the technic of roentgenologic diagnosis, there was a great increase in the number of cases in which diaphragmatic hernia was recognized. Harrington[20] stated that the most common sites of congenital hernia in the probable order of frequency are (1) the hiatus pleuroperitonealis (foramen of Bochdalek), (2) the esophageal hiatus, (3) an anterior substernal opening (foramen of Morgagni) (Larrey's spaces) and (4) the gap left by partial absence of the diaphragm, a gap which is usually situated in the posterior portion of the muscle. The sites of hernia acquired after birth are: (1) the esophageal hiatus (the type which has a hernial sac occurs here), (2) the region of fusion of the anlage of the diaphragm and (3) the aforementioned sites of the congenital type. In over 400 cases in which he operated for diaphragmatic hernia, there was not 1 case in which the hernia extended into the pericardial sac.[21]

Other than Giffin's[16] and Hedbloom's[17] mention of a diaphragmatic hernia extending into the pericardial sac no references to such an anomaly could be found in the literature. Since Hedbloom in his final report makes no reference to diaphragmatic herniation into the pericardial sac, his original mention probably referred to the defect cited by Giffin.[22] Giffin has in his notes no reference to a direct communication between the pericardial and peritoneal cavities and states that since an original article describing such a case cannot be found, we are warranted in considering ours the first to be reported.[22]

In a discussion of the development of the diaphragm, Bremer[14] stated that the most common form of diaphragmatic hernia would be that occurring through the trigonum lumbocostale, either as a true hernia forcing the fibrous tissue to bulge into the pleural cavity or as a prolapse of abdominal organs through the enclosed triangular gap, called the foramen of Bochdalek. The next most common site is in the region of the centrum tendineum on the left side near the esophagus, which is called the esophageal hiatus.

It is generally agreed that the diaphragm is formed by the septum transversum, ingrowths from the body wall, and the pleuroperitoneal membrane. The first indication of the diaphragm is the septum transversum, later forming the central tendon; the part formed by the pleuroperitoneal membrane and the ingrowths from the body wall is a later development. Embryologically, the defects most commonly observed in the diaphragm may be attributed to anomalous changes in the later stages of development.

We have been able to collect from the literature only 6 cases in which there was a defect in the diaphragm associated with a defect in the peri-

15. Riverius, cited by Harrington.[20]
16. Giffin, H. Z.: Ann. Surg. **55**:388, 1912.
17. Hedbloom, C. A.: J. A. M. A. **85**:947, 1925.
18. Hedbloom, C. A.: Ann. Surg. **94**:776, 1931.
19. Hedbloom. C. A.: Ann. Int. Med. **8**:156, 1934.
20. Harrington, S. W.: Am. J. Surg. **50**:381, 1940.
21. Harrington, S. W.: Personal communication to the authors, 1944.
22. Giffin, H. Z.: Personal communication to the authors, 1944.

cardium. In none of these were the combination and the location of defects the same as those described in our case, and in none was there a direct communication between the pericardial and peritoneal cavities. The defects of the pericardium and the diaphragm in our case we feel were the result of an early anomaly of development, which was the failure of the development of the septum transversum. The fact that the liver had dropped into an umbilical hernia further supports this explanation, since the septum transversum also contributes to the capsule of the liver and to its suspensory ligaments. We think that the congenital defects noted are the sequelae to a single developmental error at an early stage. The failure in our case to find defects in the remainder of the diaphragm makes doubtful the role of the septum transversum in the development of the remaining portions.

Perhaps it is not correct to call the elongated extrahepatic vein extending from the insertion of the hepatic veins to the inferior vena cava the ductus venosus. This is an anomalous structure and is possibly the persistent extrahepatic cephalic segment of the distal portion of the left umbilical vein. Ordinarily when the septum transversum is present, the liver develops into its substance and is prevented from migrating downward. In this instance the liver either developed at a greater distance from the heart or migrated downward during its development.

SUMMARY

A newborn infant who lived only four hours was found to present the following anomalies: (1) absence of the central tendon and of the anterior portion of the diaphragm; (2) absence of the suspensory ligament of the liver; (3) presence of a pericardioperitoneal foramen; (4) elongation of the "ductus venosus." This is the first instance of this combination of anomalies to be recorded in the literature.

Although embryologists are generally agreed that the septum transversum is the anlage of the central tendon of the diaphragm, the ventrocaudal portion of the pericardium and the suspensory ligament of the liver, no biologic proof of this fact has been afforded through the failure of its development. This case seems to offer that proof in an anomaly which occurred early in embryonic life. The elongated "ductus venosus" and the liver herniated into the umbilicus were secondary phenomena, as was possibly the dextrorotation of the ventricular portion of the heart on its venous axis.

ABERRANT PANCREATIC AND GASTRIC TISSUE IN THE INTESTINAL TRACT

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Pancreatic tissue is seldom observed outside the usual location in the duodenum. When aberrant pancreas does occur, its incidence is highest in sites close to the normal gland. The low incidence of heterotopia of the pancreas is evidenced by the small number of recorded cases. Twenty cases in which the ileum and 24 in which Meckel's diverticulum was the site have been described in the literature to date. The relative frequency of heterotopia of the pancreas in Meckel's diverticulum may be due in part to the lability of the fetal intestinal tissue during the inception of this viscus when, at about the sixth week of life, the omphalomesenteric duct is being incorporated into the umbilical group of cells. This lability is evidenced in the wide variation in size and location of this residual sac.[1] Meckel[2] discussed the clinical significance of this diverticulum in 1812 when he gave the first accurate description and the embryologic background. However, the earliest case of this diverticulum was mentioned in the literature by Lavater[3] in 1772, forty years before Meckel's description. In the Children's Hospital, Pittsburgh, during a period of sixteen years there have been 2 instances of heterotopia of the pancreas in Meckel's diverticulum and 1 of such heterotopia in the ileum. In this same period gastric mucosa has been observed in Meckel's diverticulum six times. These anomalies will be described in the following pages.

Kleb[4] first reported aberrant pancreas in 1859. In 1861 Montgomery[5] recorded 2 further cases, his own, with pancreatic tissue in the jejunum, and a case of pancreatic tissue in the ileum which had been described in the autopsy files nine years previously by Bristowe. In a survey Mitchell and Angrist[6] collected from the available literature 23 cases, including an instance of their own of pancreatic tissue in Meckel's diverticulum, and 20 cases, including 2 of their own, of pancreatic tissue in the ileum. References to these individual papers will not be repeated here.

From the Pathological Laboratories, University of Pittsburgh and the Children's Hospital.

1. Thompson, J. E.: Ann. Surg. **105**:44, 1937.

2. Meckel, J. F.: Handbuch der pathologischen Anatomie, Leipzig, C. H. Reclam, 1812, vol. 1, p. 553.

3. Lavater, cited by Curd.[9]

4. Kleb, J.: Ztschr. d. k. k. Gesellsch. d. Aerzte zu Wien **15**:732, 1859.

5. Montgomery, E.: Tr. Path. Soc. London **12**:130, 1861.

6. Mitchell, N., and Angrist, A.: Ann. Int. Med. **19**:952, 1943.

PRESENTATION OF CASES

CASE 1.—L. D., a white boy aged 6 weeks, was admitted to the Children's Hospital, Pittsburgh, July 2, 1941 with a moderately distended abdomen and a history of constipation and fever appearing thirty-six hours before admission. Administration of castor oil led to passage of greenish stools for twenty-four hours. July 2 a bloody movement of the bowel and vomiting, preceded by pain, occurred. July 3 operation revealed an intussusception with Meckel's diverticulum, which was devitalized and gangrenous. Whether the diverticulum was the leading point of the intussusception could not be determined. This intussusception was resected; a fecal fistula developed, and the child died July 15. Grossly, the pathologic specimen consisted of a pouch with an inflamed and necrotic wall and a perforation at one end. Necrosis was so widespread in the mucosa that tissue elements could not be identified microscopically. The submucosa was the site of acute inflammation and hemorrhage. The muscularis showed edema, hemorrhage and some fibrosis. External to the muscularis in the connective tissue was a nodule of pancreatic tissue containing typical pancreatic acini with granules of zymogen. No islets were identified.

CASE 2.—H. S., a 16 month old white boy, was admitted May 6, 1938 with a history of sudden onset of bloody stools and abdominal pain twenty-four hours previously. The abdomen just above the umbilicus was rigid and tender. The child was operated on May 6. Adherent to the bowel was Meckel's diverticulum, perforated at the base, and tarlike fluid was free in the peritoneal cavity. The diverticulum was resected, but the child died May 8. Grossly, no further description is available, and permission for autopsy was not obtained. Microscopic examination of the specimen showed well preserved gastric mucosa with slightly edematous submucosa and well developed muscularis mucosae. Exceptionally thick muscularis was noted in one area. Another area of the diverticulum showed an acute ulcerative process involving the mucosa and the submucosa and extending into the muscularis. Chronic cellular exudate and granulation tissue were seen in the base of the ulcer. Embedded deeply in the gastric mucosa but absent from the submucosa were groups of cells closely resembling Brunner's glands but which may have been

atypical gastric pyloric glands. Typical intestinal mucosa was present close to the areas where Brunner's glands were observed. Hemorrhage and necrosis were present beneath the intestinal mucosa at some points. On the outer side of the muscularis externa there was pancreatic tissue. Areas showing typical acinous and ductile tissue were separated by wide bands of fibrous connective tissue. Small amounts of islet tissues were also present. It is noteworthy that all elements of pancreatic, duodenal and gastric tissue were represented in this specimen.

CASE 3.—C. D., a white boy aged 3 weeks, was admitted to the hospital March 15, 1941 with a history of twenty-four hours of vomiting. Constipation was followed by a bloody stool. On admission a palpable tumor was present in the midline of the abdomen below the umbilicus. March 19 operation revealed a tumor mass in the wall of the ileum involved in intussusception. This mass was removed, but the child died March 21. Grossly, the pathologic specimen consisted of a small mushroom-shaped mass with an irregular bright red surface and a glistening white stalk. Microscopic sections contained typical pancreatic tissues with many small islets, ducts and moderate amounts of interlobular connective tissue. Necrotic debris, cellular exudate and hemorrhage were found on one surface. On the other surface there was a thin strip of smooth muscle. Some areas showed degeneration of the pancreatic tissue.

CASE 4.—W. G., a white boy 3 years of age, was admitted Aug. 30, 1942 with tenderness and rigidity of the abdomen and a palpable mass in the right lower quadrant. There was a history of recurrent attacks of abdominal cramps of eighteen months' duration which had increased in frequency from "every six weeks" to "every ten days." Some vomiting accompanied these attacks. At operation, August 31, an inflammatory mass was found in the appendical region, with fibrinous exudate on the serosal surface. A dilated cystic area was bound down by fibrinous adhesions. The child was discharged as cured September 18. Grossly, the surgical specimen consisted of a round cystic mass lined by a well developed membrane which did not communicate at the proximal end with the intestine. A sinus tract led from the interior to the surface. Microscopically, both intestinal and gastric mucosa lined the cyst wall, which contained smooth muscle and considerable amounts of fibrous connective tissue. Ulceration with acute cellular exudate, extensive fibrosis and focal necrosis were present in the wall.

CASE 5.—B. D., a white boy of 5 months, was admitted Sept. 23, 1937 with a history of inflammation about the umbilicus since birth. On admission there was an inflamed indurated area extending out from the umbilicus, fan shaped, for about one-half inch (about 1 cm.). A serous exudate was present in the umbilical pit. At operation, October 4, a large bulbous mass was

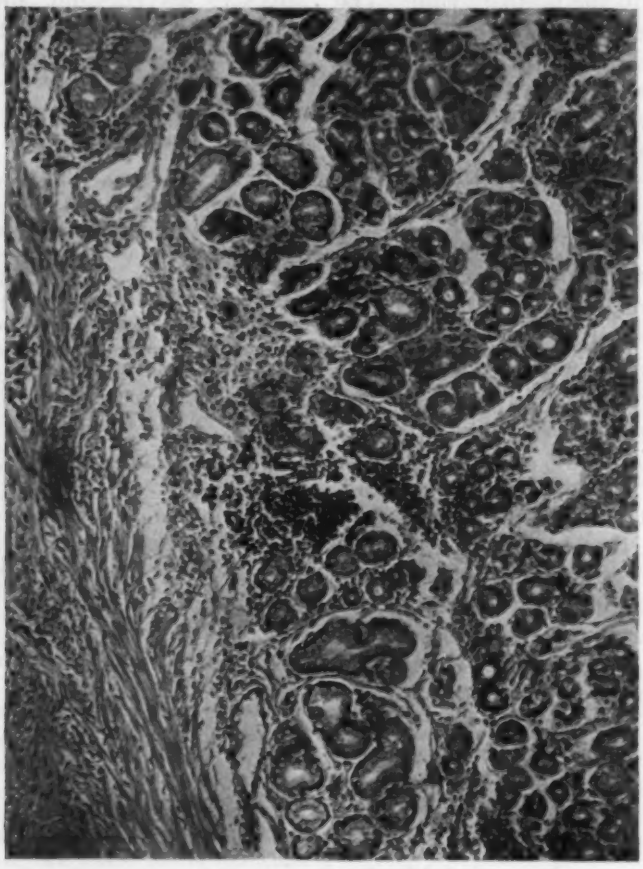

Fig. 1 (case 1).—This section reveals pancreatic tissue external to the muscularis of the diverticulum. No islet tissue was observed in the specimen. × 110.

found attached to the umbilicus and connected to the small intestine by a long sinus tract. The tract was ligated and the mass removed, but the child died October 6 with generalized peritonitis. Grossly, the pathologic specimen consisted of an ulcerated umbilicus surrounded by skin to which was attached a cystic mass embedded in granulation tissue. Microscopically, gastric mucosa was observed lining the cyst, the wall of which contained areas of fibrous connective tissue and some muscle. Vitelline duct tissue was entangled in the fibrous tissue. Ulceration with acute and chronic inflammation was present in the wall of the cyst and in the skin.

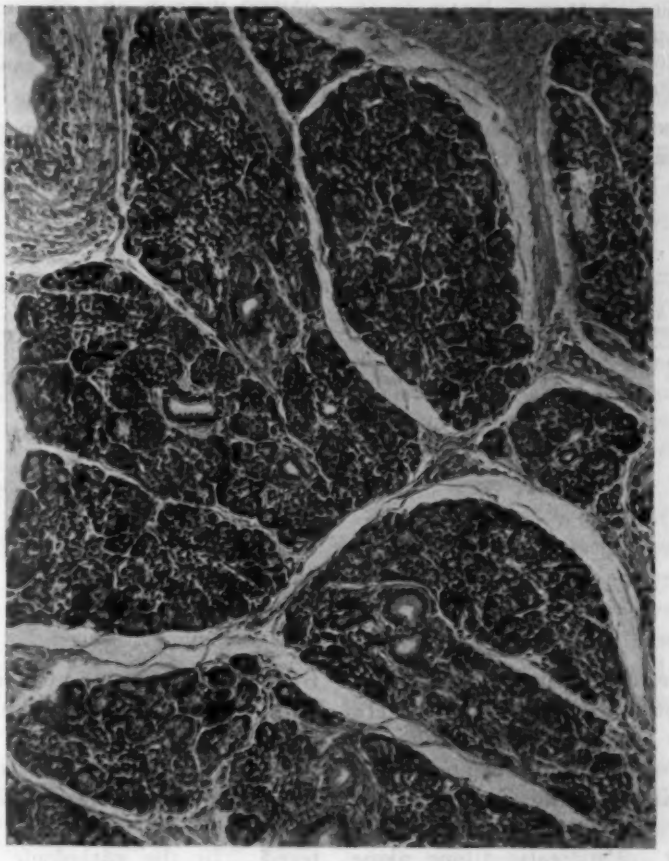

Fig. 2 (case 2).—Typical pancreatic tissue with islets of Langerhans and ducts can be seen in this section taken from the outer side of the muscularis externa of the diverticulum. × 110.

Case 6.—L. A., a white boy aged 8 years, was admitted Dec. 6, 1938 with generalized tenderness of the abdomen without muscle spasm. There was a previous history of intermittent attacks of fever, nausea and abdominal pain occurring every three to four hours for three years. Constipation had been present since birth. The day before admission the patient passed rectally a quart of old blood. December 19 operation revealed a diverticulum, 18 inches (45.5 cm.) above the ileocecal valve, extending into the mesentery. This sac was removed, and the patient was sent home December 31, after an uneventful recovery. Gross examination of the pathologic specimen revealed an indurated friable mass of tissue, the serosa of which was smooth and without exudate. It was lined by thick mucosa on which was seen one small fissure, possibly the site of the hemorrhage. Microscopically, intestinal and gastric mucosa were noted on the surface. The submucosa was edematous. Neutrophils were present in the submucosa and to a lesser degree in the mucosa. The muscularis showed some degree of parenchymatous degeneration. The serosa was edematous and congested.

Case 7.—E. B., a 5 month old white boy, was admitted Nov. 30, 1942 with a history of reddening and drainage from the umbilicus since birth. On admission a thin purulent discharge was draining from the reddened umbilicus. November 30 operation revealed an umbilical mass, which was removed successfully, and the child was discharged as cured December 15. The pathologic specimen on gross examination consisted of skin surrounding an ulcerated umbilicus and attached by a sinus tract to a piece of intestine. This "piece of intestine" proved to be Meckel's diverticulum. Microscopic examination revealed that the attached sac was lined by intestinal and gastric mucosa. Acute and chronic inflammation was present in the gastric mucosa and the skin. The submucosa and the muscularis of the sac showed varying degrees of inflammation.

Case 8.—S. W., a dehydrated Negro girl of 21 days, was admitted May 1; 1942 with a history of intermittent vomiting since birth. Visible peristalsis was present in the distended abdomen. The child died May 6. At autopsy a sac, 12 to 15 mm. in diameter, occurring about 7 cm. from the ileocecal valve, was separated

from the intestine by a thickened ligament. The wall of the sac was 4 to 5 mm. in thickness. Inflammation had extended from one end of the sac to involve the ileum and constrict its wall. This inflamed ileal wall was the site of volvulus of the intestine. Microscopically the sac was lined with gastric mucosa, some of which was disintegrating. In the better preserved areas, atrophic gastritis was present. The submucosa, the muscularis and the serosa were necrotic and contained in some areas a purulent exudate. The submucosa was distorted and partially disintegrated.

Summary.—An outline of the observations with an analysis of the clinical complications which occurred in the 8 cases is given in the accompanying table.

Three of the patients recovered and 4 died after operation. The remaining child succumbed before it could be operated on (case 8).

COMMENT

The generally accepted incidence of Meckel's diverticulum in routine autopsies is about 2 per cent, but Christie [7] has revised this figure to 1.1 per cent in a series of 5,768 necropsies. The incidence of heterotopic tissues in Meckel's diverticulum varies according to different authors. Meckel [2] reported the incidence to be 15 to 25 per cent; Kimpton and Crane,[8] 50 per cent; Curd,[9] 21 per cent, and Matt and Timpone,[10] 25 per cent. Pancreatic tissue alone occurs less frequently. Warthin,[11] reporting 47 cases of accessory pancreas, stated that it was observed in Meckel's diverticulum in 1 instance and in the ileum in 1. Hunt and Bonesteel [12] summarized the observations in 186 cases of aberrant pancreas; Meckel's diverticulum was the site of the aberrant tissue in 13 and the ileum in 13. They added 1 case of aberrant pancreas in Meckel's diverticulum. Horgan [13] observed aberrant pancreas in 2 of 314 autopsies at the Mayo Clinic, but not in Meckel's diverticulum or the ileum. In the report made by Faust and Mudgett [14] in 1940, of 370 cases of aberrant pancreas previously reported, 18 were instances of its occurrence in the ileum and 21 in Meckel's diverticulum. The Mitchell and Angrist [6] review in 1943 listed 23 cases of pancreatic tissue in Meckel's diverticulum and 20 cases of similar tissue in the ileum. One of their cases was an instance of both aberrant gastric mucosa and aberrant pancreatic tissue, resembling a case reported by Black and Packard [15] as well as a case in my series.

Although ectopic tissue may occasionally be encountered in routine operative or postmortem observations, the presence of aberrant tissue and especially of pancreas or of gastric mucosa is frequently manifested by symptoms in association with Meckel's diverticulum. The symptoms which belong to the peptic group in the classification offered by Greenblatt, Pund and Chaney,[16] namely, hemorrhage, perforation and ulceration, are those most frequently encountered. In the pathologic laboratories of the Children's Hospital hemorrhage has been observed three times, perforation three times and ulceration of the mucosa of Meckel's diverticulum four times.

Obstruction due to intussusception may be caused by Meckel's diverticulum itself or by the presence of ectopic tissue. Ectopic pancreatic tissue was the cause of intussusception in a case of my series and in those presented by Ben-

Analysis of Cases

Case	Age	Site of Aberrant Tissue: Meckel's Diverticulum	Site of Aberrant Tissue: Ileum	Type of Aberrant Tissue: Pancreas	Type of Aberrant Tissue: Gastric	Clinical Complications: Ulceration	Hemorrhage	Perforation	Intussusception	Umbilical Ulceration	Obstruction	Volvulus	Results of Operation
1	6 weeks	+	..	+	..	..	+	+	+	..	+	..	Death
2	16 months	+	..	+	+*	+	+	+	..	..	..	..	Death
3	3 weeks	..	+	+	..	..	+	..	+	..	+	..	Death
4	3 years	+	..	..	+	+	..	+	..	..	..	..	Recovery
5	5 months	+	..	..	+	+	..	..	..	+	..	..	Death
6	8 years	+	..	..	+	..	+	..	..	..	..	..	Recovery
7	5 months	+	..	..	+	+	..	..	..	+	..	..	Recovery
8	21 days	+	..	..	+	..	..	..	..	..	+	+	Patient died before operation could be carried out

* Brunner's glands were also found in this case.

7. Christie, A.: Am. J. Dis. Child. **42**:544, 1931.
8. Kimpton, A. R., and Crane, D. R.: Am. J. Surg. **49**:342, 1940.
9. Curd, H. H.: Arch. Surg. **32**:506, 1936.
10. Matt, J. G., and Timpone, P. J.: Am. J. Surg. **47**:612, 1940.
11. Warthin, A. S.: Phys. & Surg. **26**:337, 1904.
12. Hunt, V. C., and Bonesteel, H. T. S.: Arch. Surg. **28**:425, 1934.
13. Horgan, E. J.: Arch. Surg. **2**:521, 1921.
14. Faust, D. B., and Mudgett, C. S.: Ann. Int. Med. **14**:1717, 1940.
15. Black, W. C., and Packard, G. B.: Rocky Mountain M. J. **35**:859, 1938.
16. Greenblatt, R. B.; Pund, E. R., and Chaney, R. H.: Am. J. Surg. **31**:288, 1936.

jamin,[17] Ross,[18] Black and Packard,[15] Bize,[19] Brunner,[20] Hulst[21] and Taylor.[22] Aberrant gastric mucosa was the leading point of the intussusception in a case of Kimpton and Crane.[8] Inflammation of the bowel wall secondary to diverticulitis may be the cause of intestinal obstruction. This condition was found in 1 case in my series.

In 2 cases in my series Meckel's diverticulum with gastric mucosa was associated with umbilical fistula or hernia as in cases reported by Herrmann, Larson and Brown,[23] by Curd[9] and by Christie.[7]

Theories about the origin of gastric mucosa and pancreatic tissue in ectopic foci appear to take three forms: (1) that it is due to transplantation of tissue from the original site during the embryonic state, (2) that it is due to metaplasia of tissues during either embryonic or postnatal life and (3) that it is due to an atavistic phenomenon.

There have been different schools of thought concerning the types of transplantation of these tissues. Curd[9] suggested that gastric tissue became engrafted and subsequently grew in the vitelline duct. Horgan[13] assumed that during the rotation of the pancreas and its migration into the peritoneum of the posterior abdominal wall, noninflammatory adhesions pulled off small masses of pancreatic tissue, which subsequently became implanted in the area to which they were pulled. Lubarsch[24] expressed the belief that the adhesions were inflammatory in origin. Moore[25] advanced a similar theory but did not differentiate the origin of the adhesions. Zenker[26] postulated that originally there were three instead of the usual two anlages formed as the pancreas developed, that the additional anlage was carried downward as the gut elongated and that this was the source of the aberrant pancreatic tissue. Glinski[27] agreed with Zenker's view that a pancreatic anlage was carried downward, but he suggested that the source of the aberrant tissue was the ventral bud, which failed to combine with the dorsal bud. Warthin[11] believed that lateral buds from the pancreatic ducts were snared off as the ducts penetrated the intestinal wall. These lateral buds were carried down by the longitudinal growth of the intestine. Branch and Gross[28] postulated the possibilities for inclusions of primitive tissue in the foregut. Mitchell and Angrist[6] subscribed to the theory of inclusions of epithelial islands from the embryonic foregut.

Different views have been offered for the development of metaplasia. Taylor[22] regarded heterotopic tissues as dysontogenetic structures, that is, structures arising in the individual organism from abnormal differentiation of the embryonic entoderm under abnormal stimulation. King and McCallum[29] disagreed with the theories of an embryonal origin of aberrant tissue and stated that ectopic tissue develops during postnatal and more often during adult life from differentiated tissue. They postulated that inflammation leads to the metaplasia of the tissue. In support of their theory they pointed out that ectopic tissue is mingled with the tissue of the host organ and frequently occurs in those sites where inflammation is found. Most authors are not in agreement with this theory of metaplasia of adult tissue.

The third theory, that of an "atavistic phenomenon," represents a reversion of pancreatic tissues to a more primitive phylogenetic type such as that which is seen in certain lower animals and fishes. In these species pancreatic tissue is diffusely scattered through the liver, the peritoneum and the muscular coats of the intestinal wall. Mathias,[30] Beutler[31] and Delhougne[32] have advanced this theory.

The transposition theory seems to me the most acceptable because the close proximity of pancreatic and mucosal tissue to Meckel's diverticulum in an embryo having a crown-rump length of 4 mm. at the sixth week when the anlages for different tissues are being developed allows ready transfer of adjacent developing tissues. The more cephalad the point of origin of Meckel's diverticulum, the more favorable are the conditions for the implantation of aberrant tissue in this structure.

17. Benjamin, A. E.: Ann. Surg. **67**:293, 1918.
18. Ross, K.: Australian & New Zealand J. Surg. **3**:88, 1933.
19. Bize: Rev. d'orthop. **5**:149, 1904.
20. Brunner, C.: Beitr. z. klin. Chir. **25**:344, 1899.
21. Hulst, J. P. L.: Centralbl. f. allg. Path. u. path. Anat. **20**:12, 1909.
22. Taylor, A. L.: J. Path. & Bact. **30**:415, 1927.
23. Herrmann, S. F.; Larson, C. P., and Brown, B. A.: Northwest Med. **39**:377, 1940.
24. Lubarsch, O.: Virchows Arch. f. path. Anat. **254**:880, 1925.
25. Moore, R. A.: Am. J. Path. **5**:407, 1929.
26. Zenker, cited by Branch and Gross.[28]
27. Glinski, L. K.: Virchows Arch. f. path. Anat. **164**:132, 1901.
28. Branch, C. D., and Gross, R. E.: Arch. Surg. **31**:200, 1935.
29. King, E. S. J., and McCallum, P.: Arch. Surg. **28**:25, 1934.
30. Mathias, E.: Berl. klin. Wchnschr. **57**:444, 1920.
31. Beutler, A.: Virchows Arch. f. path. Anat. **232**: 341, 1921.
32. Delhougne, cited by Taylor.[22]

SUMMARY

In 3 of 8 cases of ectopic tissue described, this tissue was pancreatic tissue; in 2 of these cases it occurred in Meckel's diverticulum, and in 1, the ileum. Gastric mucosa was observed in Meckel's diverticulum six times. In 1 case in the series Meckel's diverticulum contained both types of tissue.

The 2 instances of aberrant pancreas in Meckel's diverticulum are the twenty-fourth and twenty-fifth cases to be reported. The case of pancreatic tissue in the ileum makes the twenty-first in the literature.

Of the three theories on the pathogenesis of the aberrant pancreas and gastric mucosa, the theory that the tissue was transplanted from the original site during embryonic development is believed to be the most acceptable.

EXPERIMENTAL STUDIES ON THE THERAPY AND THE PREVENTION OF DEGENERATIVE VASCULAR DISEASES

II. THE EFFECTS OF SEVERAL DETERGENTS ON EXPERIMENTAL CHOLESTEROL ATHEROMATOSIS OF RABBITS

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In previous publications (Hueper [1]) attention was called to the fact that colloidal phenomena play an important role in the production and in the prevention of atherosclerosis. Among the various agents naturally occurring in the plasma and exerting a definite influence on the colloidal stability and the dispersion of cholesterol and on its penetration of, and status in, filtration membranes are lecithin, fatty acids, proteins and bile acids (Schönheimer [2]; Joël and Schönheimer [3]; Klinke [4]; Höber and Höber [5]; Loeffler [6]; Fieser [7]; Wieland and Sorge [8]). Certain experimental investigations (Page and Bernhard [9]; Malisoff; Hueper [1b]; Chaiika [10]) suggest that diiodide ricinoleate, oleic acid and potassium thiocyanate given orally to cholesterolized rabbits interfere with the production of atheromatous deposits through an effect on the colloidal equilibrium of the plasmas lipoids.

Industry has developed during recent years a large number of synthetic agents with surface-active properties which display similar qualities in relation to lipoidal material. According to their predominant electrical charges, they are divided into three types—anionic, cationic and nonelectrolytic—which differ from each other in chemical structure and composition and in compatibility with other substances and with environmental reactive conditions (Morris [11]). The anionic detergents are saponified vegetable oils or soaps, sulfonated aliphatic esters, sulfated alcohols and sulfonated aliphatic ethers (Van Antwerpen [12]). The cationic detergents are quaternary ammonium derivatives, occurring also in the form of piperidine, betaine and phosphonium compounds. All have to varying degrees the properties of lowering surface tension, lowering interfacial tension, increasing wetting power, deflocculating and dispersing suspended matter, exerting a detergent effect by solubilization and sorption, and acting to produce foam. They consist of a polar or hydrophilic group and a nonpolar or hydrophobic group.

The use of synthetic surface-active agents has reached considerable proportions in industry, where they are employed in the dyeing, sizing, finishing and scowing of textiles, in degreasing, in the preparation of soapless shampoos and other substitutes for soap, of dentrifices, of wall paper removers, and for many other purposes. Because of their germicidal properties, the cationic detergents are utilized for the disinfection of food containers in restaurants and hotels, of tanks, pipelines and bottles in the milk industry, of linen and clothes in laundries, and of floors in buildings and ships. Because of these properties, detergents have recently entered the medicinal field, where they are used in the sterilization of the skin and of instruments, in the treatment of mastitis of cows, in the cleansing of burns, in the control of gastroduodenal ulcers because of their inhibiting effect on the peptic action of the gastric juice and in the composition of ointments to increase the permeability of the skin and thus facilitate penetration of therapeutic

From the Warner Institute for Therapeutic Research.

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agents (Rosenberg[13]; Green[14] Herrmann[15]; Sulzberger and Baer; Fogelson and Shoch[16]; Bull and Neurath[17]; Epstein, Throndson, Dock and Tainter[18]; Hatton, Fosdick and Salandra[19]; Scales and Kemp[20]; Duemling[21]; Sluhan[22]; Snell[23]; Smith[24]; Hatch and Rice[25]; Caryl and Ericks[26]; Morris[11]; Fani and Snell,[27] and others).

The experiments to be reported represent some exploratory studies designed to determine the potentialities of these agents in the prevention and the therapy of atherosclerosis. The experiments fall into two groups: the first group comprises studies in which solutions of several detergents were introduced either orally or intravenously into normal rabbits with a view to ascertaining whether or not detergents alone might elicit in normal animals atheromatous or other abnormal vascular lesions by changing the colloidal equilibrium of the plasma lipoids or by altering the permeability of the vascular walls. In the second group the effects of detergents on cholesterolized rabbits were studied.

EXPERIMENTS ON NORMAL RABBITS

Oral Administration.—Three groups of 2 rabbits each were fed daily by stomach tube 10 cc. amounts of 1 per cent aqueous solutions of the following three detergents, respectively: Tergitol Penetrant 08, Triton K-60 and Nacconol FSNO. Tergitol Penetrant 08 is the sodium salt of a higher primary alkyl sulfate having the formula $C_4H_9CH(C_2H_5)CH_2SO_4Na$, manufactured by Carbide and Carbon Chemical Corporation. Nacconol FSNO is a sodium alkylaryl sulfonate made by the National Aniline & Chemical Co., Inc. These surface-active agents are anionic substances. Triton K-60 is cetyldimethyl benzylammonium chloride, a cationic detergent produced by Röhm & Haas Co., Inc.

The 2 rabbits given Tergitol showed no significant changes in amount of hemoglobin, numbers of erythrocytes and leukocytes, hematocrit values, plasma viscosity or sedimentation rate during the experimental period. This lasted eleven days for the first rabbit; the second was killed on the twentieth day. The levels of total serum cholesterol and cholesterol ester remained stationary in both rabbits during these periods. Shortly before death diarrhea developed in both animals. One rabbit at postmortem examination had a turbid serous fluid in both pleural cavities, and both rabbits had congestion of the lungs. The gastrointestinal tract of each was hyperemic. All other organs were grossly normal.

The histologic study of the organs of this group revealed hyperemia of the liver associated with a fine granular state of the cytoplasm of the liver cells. The large branches of the pulmonary artery exhibited subendothelial edema and duplication or crowding of the endothelial cells. One aorta revealed necrosis of the inner media with calcification of the fibrillar structures; the structures adjacent to the calcified plates were edematous (fig. 1 *A*). A similar medial change was present in the ascending portion of the second aorta, which also showed moderate round cell infiltration in the degenerated area.

In the 2 rabbits given Nacconol diarrhea also developed. One died on the sixth day of the experiment, and the second was killed on the twentieth day. The latter showed considerable hemoconcentration at the day of death, with an approximate 30 per cent increase in amount of hemoglobin, number of erythrocytes and volume of packed cells. There were no appreciable changes in number of leukocytes, viscosity of plasma or sedimentation rate. The values for total serum cholesterol and cholesterol esters remained within the normal range. At autopsy the right pleural cavity of the one rabbit contained a greenish yellow fluid, and that of the other a slightly bloody serous fluid. The lungs were hyperemic. The adrenal glands were small and faintly grayish green. All other organs were normal.

On histologic examination the lungs of both rabbits revealed marked hyperemia, edema and intra-alveolar hemorrhages. In the lungs of 1 animal these changes were associated with purulent pleurisy, cellular thickening of the interalveolar septums and marked proliferation of the epithelial lining of the bronchi, complicated in places by ulcerations of, and hemorrhages into, the bronchi. In the solidified pulmonary tissue surrounding such bronchi, strands and groups of atypical irregularly shaped and sized epithelial cells were seen diffusely invading the granulomatous tissue (fig. 1 *B*). The pulmonary vessels were normal. The livers of both animals were hyperemic, and one of them showed atrophic liver cell cords and hemorrhages into bile ducts. Both aortas revealed multiple foci of subintimal medial necrosis and calcification (fig. 1 *C*). Other organs were essentially normal with the exception of one kidney, which was markedly congested and contained extensive wedge-shaped mononuclear infiltrations surrounding cystically distended tubules filled by occasional calcium casts.

The 2 rabbits given Triton died twelve days after the start of the experiment. The blood showed no consistent or significant fluctuations in amount of hemoglobin, numbers of red cells and white cells, volume of packed cells, sedimentation rate, plasma viscosity or amounts of total serum cholesterol and cholesterol esters. At autopsy the pleural cavities of both rabbits contained a bloody serous fluid. Some fibrinous deposit covered the pleural linings. The livers were red-brown with yellow patches.

13. Rosenberg, N.: Surgery **13**:385, 1943.
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18. Epstein, S.; Throndson, A. H.; Dock, W. M., and Tainter, M. L.: J. Am. Dent. A. **26**:1461, 1939.
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27. Fani, J. M., and Snell, F. D.: Indust. & Engin. Chem. (Indust. Ed.) **31**:48, 1939.

Histologic study showed the lungs to be congested, edematous and focally hemorrhagic. Purulent bronchitis was present in both animals. One of them had a bronchial lining that consisted of densely packed hyperchromatic cells of round or oval shape and indistinct outline. The peribronchial tissue was composed of cellular fibroblastic tissue containing numerous eosinophilic leukocytes. At the border zone between the granulomatous peribronchial tissue and the pulmonary parenchyma numerous irregular glandular formations lined by one to several layers of hyperchromatic small cells were found invading both the granulomatous tissue and the pulmonary parenchyma. Both aortas were normal.

Intravenous Administration.—Two rabbits received daily intravenous injections of 5 cc. of a 0.1 per cent solution of Nacconol NRNO (sodium alkylaryl sulfonate, made by the National Aniline & Chemical Co., Inc.). Three rabbits were given intravenous injections of 5 cc. of a 0.1 per cent solution of Triton NE (arylalkyl polyether alcohol, made by Röhm & Haas Co., Inc.). Two rabbits received daily intravenous injections of 5 cc. of a 0.033 per cent solution of Aerosol OT (dioctyl ester of sodium sulfosuccinic acid, made by the American Cyanamid & Chemical Corporation). The solvent for each of the detergents named was isotonic solution of sodium chloride. While Nacconol NRNO and Aerosol OT are anionic detergents, Triton NE is of the neutral nonionic type. It was ascertained by preliminary tests that amounts exceeding 20 cc. of the solutions when intravenously injected into rabbits elicited spastic convulsions with marked meningism, associated

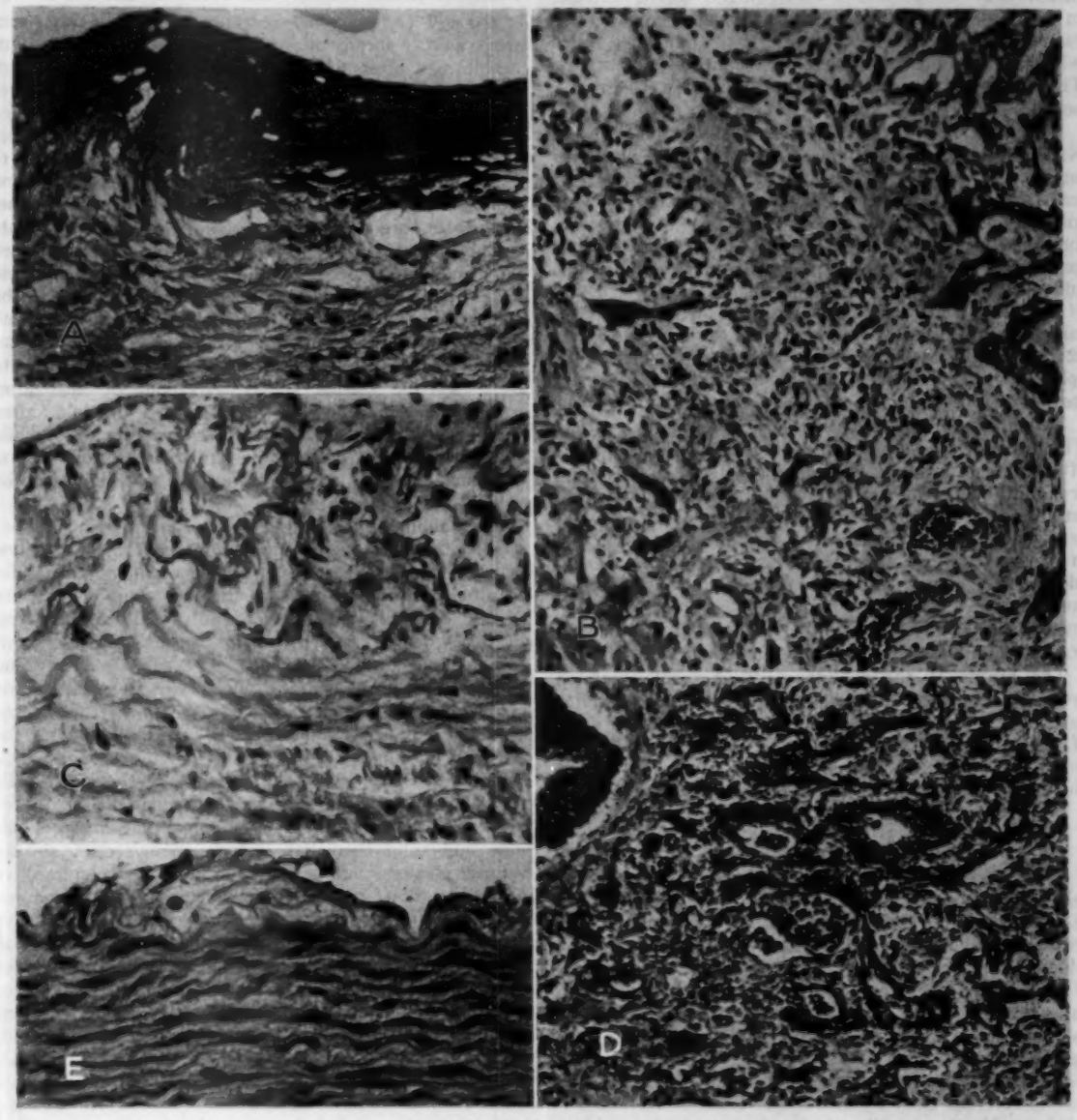

Fig. 1.—*A*, subintimal calcification with edema of the surrounding media of the aorta. *B*, granulomatous tissue in the lung with solid carcinomatoid epithelial cell strands. *C*, liquefaction necrosis and calcification of the inner media of the aorta. *D*, granulomatous pulmonary tissue with small cell hyperplasia of the bronchial epithelium and proliferation and invasive growth of atypical carcinomatoid glandular formations. *E*, early atheromatous lesion of the aorta of the edematous, homogeneous type.

with hemorrhages in the lungs, the subepicardial tissue and the thymus, ending in death. One of the rabbits given Nacconol died after twenty-four days; the second, after fifty-six days. The rabbits given Triton died after ten, twenty-four and fifty days, respectively. The rabbits given Aerosol were put to death after sixty-one days. After several injections the ears of the rabbits became swollen, red and finally necrotic at the sites of injection, with large defects resulting.

The rabbits given Nacconol showed at autopsy hemorrhagic lungs and a blood-tinged serous exudate in the pleural cavities. The rabbits given Triton exhibited congested and cyanotic small intestines, filled with watery fluid, and hyperemic meningeal membranes. The organs of the rabbits given Aerosol were grossly normal.

The histologic examinations of the rabbits given Nacconol showed the lungs congested and edematous and the livers hyperemic and edematous. One liver was studded with numerous necroses and leukocytic infiltrations. The brains of both rabbits revealed numerous perivascular lymphocytic accumulations, congested vessels, with partly hyalinized and swollen walls, and many small necroses in the cortex with destruction of ganglion cells. The kidneys of both rabbits contained tubular degenerations, and those of 1, multiple abscesses. The other organs (aorta, stomach, intestine, spleen, adrenal glands, heart, bone marrow) were normal.

The brains of the rabbits given Triton were normal. The lungs were hyperemic and edematous, and those of 2 rabbits contained hemorrhagic foci and purulent bronchitis with leukocytic infiltration of the surrounding interstitial tissue, including the hyperplastic walls of arteries. A hemorrhage into an aortic leaflet was found in the heart of 1 rabbit. The livers were congested and edematous. Hemorrhages were seen in the intestinal mucosa and in the intestinal lumen, as well as in the mucosa of the urinary bladder, of 1 rabbit. All other organs of both rabbits, including the aorta, were normal.

The brains of the rabbits given Aerosol exhibited arterioles with swollen and hyaline walls. Similar vascular changes were found in the kidneys, where some of the arterioles were completely obliterated by the hyaline thickening of the walls. All other organs, including the aorta, were essentially normal.

EXPERIMENTS ON CHOLESTEROLIZED RABBITS

A series of 30 rabbits divided into five groups received daily 0.5 Gm. of cholesterol dissolved in 12 cc. of cottonseed oil and mixed with a small amount of powdered rabbit chow. The first group of 6 rabbits, which served as a control, did not get any other type of treatment. A second group of 6 rabbits was given daily, by injection in an aural vein, 5 cc. of a 0.05 per cent solution of Aerosol OT dispersed in a 0.075 per cent solution of methylcellulose (4,000 centipoises) in isotonic solution of sodium chloride. A third group of 6 rabbits received daily by mouth 0.25 Gm. of Aerosol OT in about 20 cc. of milk. A fourth group of 6 rabbits was fed daily 0.25 Gm. of Triton NE in milk. The fifth group of 6 rabbits was given daily by mouth 0.002 Gm. of lithium iodide plus 0.006 Gm. of potassium thiocyanide in water. After one week the dose of lithium iodide was increased to 0.05 Gm. daily. The rationale of the last-mentioned combination of chemical agents is based on the consideration that potassium thiocyanide apparently prevents cholesterol atheromatosis by an influence on the colloidal equilibrium of the plasma (Hueper [1b]) and at the same time suppresses thyroid function. It appeared desirable to offset the latter effect by the simultaneous administration of an iodide. Lithium iodide was selected for this purpose as it also acts to render soluble in an aqueous medium lipoid-soluble substances such as aniline and toluene and behaves in this respect similar to bile acids (McBain [28]).

For all rabbits determinations were made once a month of the following: hemoglobin, erythrocytes, leukocytes, erythrocyte sedimentation, plasma viscosity, total serum cholesterol and ester cholesterol. Blood for these studies was withdrawn from the jugular vein. For histologic examination the aorta was either cut into twelve to fifteen rings or was rolled up and cut in toto.

Aerosol OT Injected Intravenously.—The injections were neither locally nor generally well tolerated. One rabbit died after fifteen days, a second after nineteen days, a third after twenty-three days and a fourth after thirty-six days. The 2 remaining survivors were killed after sixty-eight days when it became impossible, because of inflamed ears and thrombosed veins, to continue the intravenous injections. There were no significant changes in numbers of erythrocytes and leukocytes, amount of hemoglobin, sedimentation rate or plasma viscosity. The total cholesterol increased only moderately in 1 of the 2 surviving rabbits and never rose above twice the original figure, while at the same time the ester fraction decreased toward the end of the experimental period so that the ratio of total cholesterol to ester cholesterol dropped from 3:1 to 8:1. In the second surviving rabbit, on the other hand, the total cholesterol increased fivefold after three weeks and eightfold after two months, while the ratio of total serum cholesterol to the ester fraction remained approximately constant. The weights of the rabbits remained stationary.

The rabbits which died showed edematous and hemorrhagic lungs. One of them, moreover, had yellow necroses in the liver and a subserous hemorrhage extending from the pylorus to the duodenum. The ears of the 2 rabbits which were put to death were extensively necrotic and the aortas were wide and yellow.

The histologic examination revealed edema and hemorrhages in the lungs and brain and edema and congestion of the liver of every rabbit in this group. In the rabbit which died on the nineteenth day of the experiment the bronchial epithelium formed a thick multilayered lining of small oval hyperchromatic cells in palisade arrangement. The surrounding loose fibroblastic inflammatory tissue contained numerous glandular and solid epithelial strands composed of similar cellular elements and permeating diffusely the connective tissue matrix (fig. 1 *D*). The liver exhibited numerous large hyaline necroses of the liver cells with extensive cellular as well as diffuse calcifications. There was also an appreciable increase of the interstitial connective tissue, which in places contained considerably proliferated bile ducts. The aorta of this rabbit revealed in the thoracic part extensive medial calcifications with thinning of the aortic wall. In none of the animals was there any evidence of a foam-cellular and homogeneous thickening of the intima of the aorta and the large arteries. In the 2 surviving rabbits, however, there was a foam-cellular swelling of the cells of the adrenal cortex. All other organs were normal.

Aerosol OT Given Orally.—The oral treatment with Aerosol OT and cholesterol was continued for five months. During this period there occurred no significant fluctuations in numbers of red cells and white cells, amount of hemoglobin, sedimentation rate or plasma

28. McBain, J. W.: Solubilization and Other Factors in Detergent Action, in Kraemer, E. O.; Bartell, T. E., and Kistler, S. S.: Advances in Colloid Science, New York, Interscience Publishers, Inc., 1942, vol. 1, p. 99.

viscosity. During the first month the total serum cholesterol increased in general more rapidly than the ester fraction. Two months after the start of the experiment the original ratio of 1.5:1 to 3:1 was reestablished, and from then on to the end of the experiment the values of total cholesterol ranged between 300 and 400 mg. per hundred cubic centimeters while the ester values moved between 125 and 280 mg. per hundred cubic centimeters. The weights of the rabbits increased progressively.

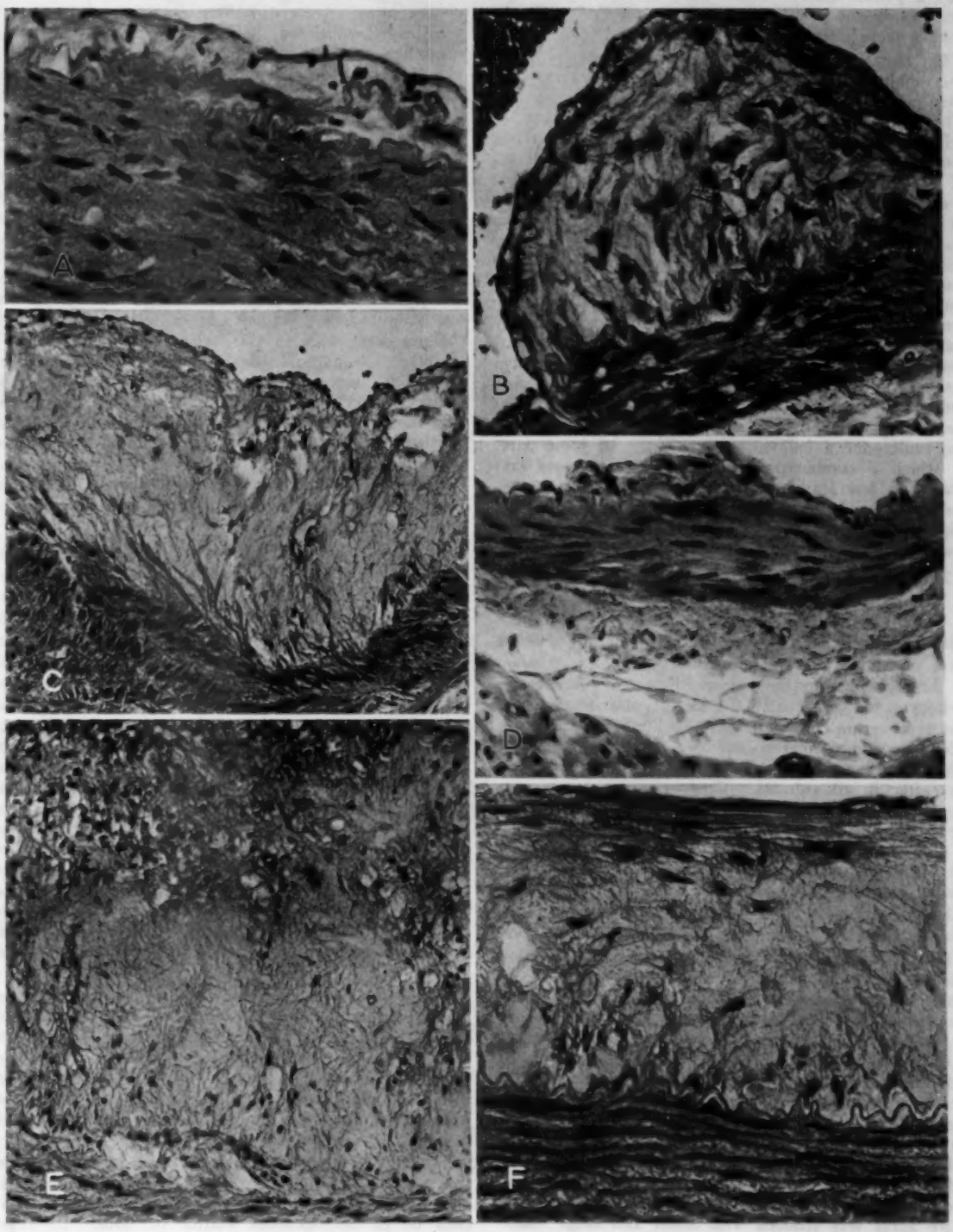

Fig. 2.—*A*, subintimal edematous swelling with scattered foam cells. *B*, foam-cellular and histiocytic intimal thickening in a coronary artery. *C*, well developed homogeneous type of aortic atheromatosis showing fibroblasts invading the base of the lesion from the media. *D*, early foam cell transformation and proliferation of the endothelial cells in a myocardial artery. *E*, foam-cellular and histiocytic intimal cushion of the aorta with multiple foci of homogenization and fibroblastic proliferation. *F*, edematous intimal thickening of the aorta covered by several layers of smooth muscle cells.

One of the rabbits died four months after the start of the experiment. The 5 survivors were killed after five months. The rabbit which died had a cheesy exudate in the pleural cavities and a serous fluid in the abdomen. The lungs were collapsed, and the liver had a yellow color. There were no gross pathologic changes in the 5 rabbits which were killed. All the rabbits were obese.

On histologic examination the adrenal glands of all the rabbits showed a foam-cellular swollen cortex. The liver cells had mildly to moderately granular cytoplasm. The aortas of 4 rabbits had minor to moderate foam-cellular intimal thickenings involving only the ascending portion except in some rabbits in which a few rings of the thoracic part were also involved. In one ring a calcification of the inner media was seen in an area in which the intima was normal. The abdominal portion was normal. In the aortas of the remaining 2 rabbits foam-cellular and homogeneous intimal cushions were found throughout, but did not involve all rings. There was definite evidence of a direct foam cell transformation of the endothelial cells. In some places the early stage of atheromatosis was characterized by an edematous localized swelling of the subendothelial intima, often associated with a swelling and increase of the overlying endothelial cells (fig. 1 *E*). In other foci individual endothelial cells or smaller or larger groups of them had undergone a balloon-like swelling. In some parts in which a combination of these two processes was present, a few foam cells covered more or less homogeneous deposits containing a few oval-shaped nuclei (fig. 2 *A*). The other organs were normal (thyroid, heart, pancreas, intestine, kidney, uterus, spleen).

Triton NE Given Orally.—The oral administration of Triton NE was continued for five months, at the end of which the rabbits were killed by an intravenous injection of a 4 per cent formaldehyde solution. There were no significant and consistent changes in amount of hemoglobin, number of erythrocytes, number of leukocytes or sedimentation rate of plasma viscosity. The total serum cholesterol increased uniformly after one month to approximately 350 mg. per hundred cubic centimeters and remained between 400 and 500 mg. thereafter in all animals. The ratio of the esters to the total cholesterol was in all rabbits about 1:3 at the beginning of the experiment and soon dropped to 1:2, indicating that the free cholesterol fraction did not become elevated as much as the ester fraction. The rabbits gained progressively in weight.

The rabbits were obese at autopsy. All organs were grossly normal.

The histologic examination showed the pulmonary arteries of 3 rabbits and the pulmonary veins of 1 rabbit involved by foam-cellular cushions. In 1 rabbit the media also was invaded to an extensive degree. In smaller pulmonary arteries and arterioles there were proliferation and foamy swelling of indistinctly outlined endothelial cells covering a narrow hyaline necrotic zone extending into a swollen media. Only one of the lungs contained foci of foam cells in its parenchyma. One extramyocardial coronary artery displayed a small foam-cellular intimal cushion (fig. 2 *B*). Large foam-cellular and homogeneous intimal deposits were present in the sinuses of Valsalva. In 4 rabbits the intima of the entire aorta was studded with small and large foam-cellular and homogeneous cushions (fig. 2 *C*). Moderate changes of this type were present in the other 2 rabbits of this group. The early lesions exhibited either a marked and almost plump cylindric swelling of the endothelial cells covering the inside of the aorta in single file or small elevations beneath which a homogeneous matter was present in the subintimal space. In some of these foci the foamy balloon-like swollen endothelial cells projected into the aortic lumen (fig. 2 *D*). In other areas small circumscribed swellings in the intima had been produced by the homogeneous material in the intima and the subintimal space. Some of the more advanced lesions consisted of masses of foam cells, which in the deeper parts had often degenerated into homogeneous areas with shadows of large oval nuclei, fibroblasts and, occasionally, star-shaped monocytes (fig. 2 *E*). In a few cushions the degenerated central mass was covered by several layers of smooth muscle cells which in turn were lined in places by some endothelial cells with foamy cytoplasm (fig. 2 *F*). Other lesions apparently from the start were composed almost exclusively of a homogeneous material containing a few oval nuclei. Normal endothelial cells often covered such foci. The media was usually intact. In 2 rabbits numerous calcifications and necroses were present in this layer and were not always associated with atheromatous lesions in the overlying intima. Similar and extensive atheromatous changes were seen in the large aortic branches of 2 rabbits. The adrenal glands presented in all instances a swollen, foam-cellular cortex. The cytoplasm of the enlarged liver cells had a fine granular appearance. The medium-sized arteries of the spleens of 2 rabbits consisted of loose vacuolated media lined by crowded endothelial cells. The renal arteries of 2 rabbits contained small foam-cellular intimal cushions. The other organs were essentially normal (heart, thyroid, pancreas, uterus, ovary).

Lithium Iodide–Potassium Thiocyanide Given Orally.—The experiment was continued for five months, at the end of which 5 rabbits were put to death by an intravenous injection of a 4 per cent formaldehyde solution. One of the rabbits died two months after the start of the experiment. There were no significant changes in amount of hemoglobin, numbers of erythrocytes and leukocytes, sedimentation rate or viscosity of plasma during the experimental period. The total cholesterol increased within one month to around 400 mg. per hundred cubic centimeters from an original level of 65 to 100 mg. During the remainder of the experiment the values fluctuated around 500 mg. The cholesterol ester rose from an original level of about 30 to 35 mg. to 150 to 250 mg. after one month and fluctuated between 200 and 300 mg. thereafter. The ratio of ester cholesterol to total cholesterol, which initially was 1:3 dropped to 1:2 during the latter part of the experimental period. The livers were slightly granular and brown-red at autopsy. All other organs were without abnormalities.

The histologic examination showed large and partially cystic thyroid follicles filled with a solid pink-stained colloid. The lung of 1 animal contained foam cell infiltrations of the interalveolar network. The myocardial arteries of 2 rabbits revealed in the first instance homogenization of the media and in the second instance foam-cellular intimal cushions infringing considerably on the vascular lumen (fig. 3 *A*). The ascending portion of the aorta, as well as all or most of the rings cut from the thoracic and abdominal portions, exhibited extensive intimal cushions composed of foam cells or of homogeneous matter or both. In some instances these deposits extended into the media. Similar lesions were found in the large aortic branches of 2 rabbits. No intimal changes were noted in the aorta and other arteries of the rabbit which died two months after the start of the experiment. The livers contained foamy liver cells. The adrenal cortex in

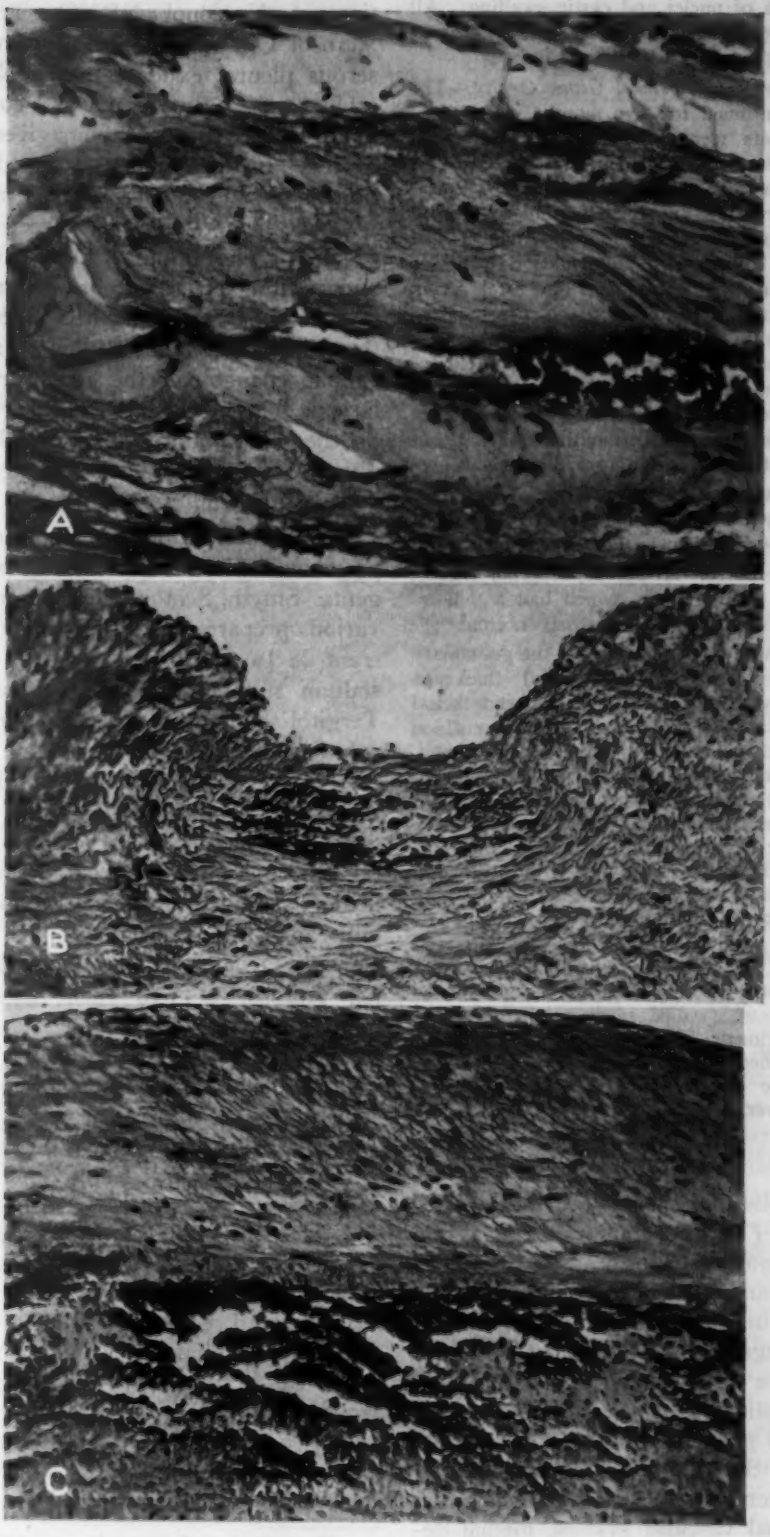

Fig. 3.—*A*, myocardial artery with homogenization of the media involving approximately one half of the circumference of the vessel. *B*, medial calcification situated between two foam-cellular atheromatous intimal cushions of the aorta. *C*, extensive calcification at the base of an old, partly homogeneous atheromatous intimal cushion in the aorta.

every instance was composed of large foam cells which showed in part loss of nuclei and cystic swelling. All other organs (heart, spleen, pancreas, ovary, uterus, parathyroid glands) were essentially normal.

Cholesterol Control: Cholesterol Given Orally.—The experiment was continued for five months, at the end of which the rabbits were killed by an intravenous injection of a 4 per cent formaldehyde solution. One rabbit died seven weeks after the start of the experiment. There were no appreciable and significant fluctuations in amount of hemoglobin, numbers of erythrocytes and leukocytes, sedimentation rate or plasma viscosity during this period. The total serum cholesterol rose to approximately 250 mg. per hundred cubic centimeters within the first month of the experiment and then advanced gradually to somewhat above 400 mg. during the third and fourth months. It dropped thereafter to between 200 and 300 mg. In 1 instance it was reduced during the fourth month to 47 mg. but rose during the last month again to 178 mg.

The ratio of cholesterol esters to total cholesterol remained usually within limits of 1:2 to 1:3. At times, however, it went down as low as 1:7.

The animals were obese. At autopsy internal organs, with the exception of the livers, which had a yellow-brown color, were observed to be grossly normal.

The histologic examination showed that the pulmonary arteries in the lungs of 1 rabbit had thickened vacuolated walls with an occasional subendothelial hyalinization. A large intimal foam-cellular cushion was present in a coronary artery of a second rabbit. In 4 rabbits the aorta exhibited foam cell and homogeneous cushions throughout its entire length, while in 1 rabbit these changes were restricted to the ascending part. In 2 animals of the first-mentioned group calcifications of the intima or the media were met with in several rings studied (figs. 3*B* and *C*). The livers were composed of hepatic cells with foamy cytoplasm. The adrenal cortex in each instance was swollen and consisted of foam cells. The other organs were normal. There were no atheromatous lesions in the aorta of the rabbit which died seven weeks after the start of the experiment. The small renal arteries, however, had calcified walls, while the tubules were degenerated. There were extensive necroses present in the edematous liver.

COMMENT

In normal rabbits the repeated oral administration of detergents (Tergitol Penetrant 08; Triton K-60 and Nacconol FSNO) produced diarrhea in the majority and elicited in 5 of 6 rabbits pleural effusion in addition to hyperemia and edema of the lungs. The pulmonary condition in 2 rabbits (given Triton and Nacconol, respectively) was complicated by the development of atypical epithelial proliferations of a carcinomatoid character in the granulomatous pulmonary parenchyma. Whereas the Triton-treated rabbits exhibited a normal vascular system, medial degenerations and calcifications were found in the aortas of the rabbits which ingested Tergitol and Nacconol. Edematous and endothelial proliferative lesions were present in the pulmonary arteries of the rabbits given Tergitol.

The prolonged and repeated intravenous injection of Nacconol NRNO, Triton NE and Aerosol OT resulted in the development of a serous pleural exudate in the Nacconol-treated rabbits only. But edema and congestion of the lungs occurred in the rabbits given Nacconol and Triton. In the Triton-treated rabbits the irritation of the intestinal tract was most prominent. Multiple hemorrhages were seen in the internal organs of these animals. Hyaline degeneration and swelling of the cerebral arteries were noted in the rabbits treated with Nacconol and those treated with Aerosol and were associated with similar changes in the renal arterioles of the latter. The aorta and the other large arteries, on the other hand, were normal. The rabbits given Aerosol had no other significant abnormalities in their internal organs.

These observations confirm in part those of previous investigators on the toxicity of detergents. Smyth, Seaton and Fischer,[29] who studied various preparations of Tergitol (Tergitol Penetrant 08 [a 40 per cent aqueous solution of the sodium sulfate derivative of 2-ethyl hexanol], Tergitol Penetrant 4 [a 25 per cent aqueous solution of the sodium sulfate derivative of 7-ethyl-2-methyl undecanol-4] and Tergitol Penetrant 7 [a 25 per cent solution of the sodium sulfate derivative of 3,9-diethyl tridecanol-6]) on rats and guinea pigs found congestion and hemorrhage of the stomach, necrosis of intestinal villi and minor degenerations of the liver. Benaglia, Robinson, Utley and Cleverdon,[30] who fed Aerosol OT to rats, dogs, rabbits and monkeys, observed the appearance of diarrhea in rabbits and rats. There were no changes in the various blood constituents or in the histologic structure of the internal organs. It may be mentioned briefly that Macht[31] on injecting a solution of sodium lauryl sulfate subcutaneously and intramuscularly into rabbits, guinea pigs, rats and mice observed necrotic changes at the sites of injection and on instilling the same agent into the conjunctival sacs of cats and rabbits noted irritation and reddening. The intravenous injection of this sulfate as a 0.01 per cent solution produced a transitory fall in blood pressure. Höber and Höber,[5] who transfused the isolated liver of a frog with Aerosol OT and MA, noted that a detergent added to a serum containing transfusion fluid accentuated the action of the

29. Smyth, H. F.; Seaton, J., and Fischer, L.: J. Indust. Hyg. & Toxicol. **23**:478, 1941.

30. Benaglia, A. E.; Robinson, E. J.; Utley, E., and Cleverdon, M. A.: J. Indust. Hyg. & Toxicol. **25**:175, 1943.

31. Macht, D. I.: Federation Proc. **3**:30, 1944.

serum in regard to the incorporation of colloidal dyes into the stellate cells which became swollen by hydration. High concentrations of detergent in the transfusion fluid induced cytolysis by unfolding or disrupting the protein molecules, thereby causing their denaturation.

The present studies add the fact that the five tested anionic detergents when given orally or intravenously produced degenerative and in part also calcifying lesions in the walls of the large or the small arterial vessels, while the only cationic detergent (Triton K-60) used had no such effect. Moreover, it is remarkable that carcinomatoid proliferations occurred in the lungs of 2 rabbits after the oral introduction of detergents (Triton K-60, Nacconol FSNO). This observation may not be entirely incidental, as similar changes were found in the lung of a cholesterolized rabbit given intravenous injections of Aerosol OT. The mechanism of these lesions, however, is obscure. The various untoward effects of detergents are attributable in part to their denaturing action on proteins with which they undergo interreactions (Putnam and Neurath [32]; Kuhn and Bielig [33]); in part they may be connected with a disorganizing effect on the lipoids and lipoproteins, particularly those located in the cellular and filtration membranes (Baker, Harrison and Miller [34]), resulting in severe changes in the permeability of these tissues.

In the cholesterol-fed series there did not occur any significant hematic changes except those related to the cholesterol content of the serum. It is noteworthy here that the total cholesterol content was lowest in the groups of rabbits which received injections of, or were fed, Aerosol OT. The increase in the serum cholesterol of these animals during the early period of the experiment was mainly due to an elevation of the level of free cholesterol. A more regular and appreciable response of the cholesterol content of the serum to the alimentary introduction of cholesterol was exhibited by the control series, which showed also the typical wave reaction in the elevation of the level of serum cholesterol. The quickest, highest and most persistent increases in serum cholesterol were displayed by the rabbits receiving Triton NE and lithium iodide plus potassium thiocyanide; in these groups the cholesterol level rose within one month to 300 to 400 mg. per hundred cubic centimeters and remained later between 400 and 500 mg. without showing any tendency to drop secondarily to lower figures as did regularly that in rabbits fed cholesterol only.

The severity and distribution of the atheromatous arterial lesions in the different groups in general paralleled these differences in the cholesterol content of the blood. In the group given Aerosol intravenously, the arterial changes were restricted to the occurrence of medial degenerative and calcifying lesions in the aorta of 1 rabbit, while in the group given Aerosol orally such lesions appeared in association with mild to moderate athermatous reactions limited mainly to the ascending portion of the aorta. No other vessels were affected. In the groups treated with Triton NE and lithium iodide–potassium thiocyanide and in the cholesterol control group, on the other hand, marked and diffuse atheromatous responses were found as a rule not only in the entire aorta but in other vessels, such as the pulmonary, carotid, iliac, coronary and renal arteries. These lesions were found most pronounced in the two groups treated with Triton NE and lithium iodide–potassium thiocyanide, which had the highest and most persistent elevation of serum cholesterol values. It seems likely, therefore, that these agents not only favored the absorption of cholesterol from the intestinal lumen, and thereby created a more marked, persistent and rapid increase of the serum cholesterol, but enhanced the deposition of the lipoid in the vascular wall. This atheromatogenic action of lithium iodide is apparently not impaired by the simultaneous administration of potassium thiocyanide, although the effectiveness of the latter substance on the function of the thyroid gland was evidenced by the large cystic follicles filled with solid colloid. Inasmuch as the concentrations of the detergents in the blood were not determined, it remains uncertain whether or not the reported effects on the lipoidal blood constituents and the vascular walls are of a direct or an indirect character. They demonstrate, however, that the various detergents differ in their influence on these constituents. Future investigations may show definite relations between the chemical structures and the types of detergents and their effects on atheromatogenesis.

The observations made on the histogenesis of atheromatosis confirm again the concept previously advanced that the lipoidal material enters the vascular wall either through being incorporated into the endothelial cells, thereby giving rise to the development of endothelial

32. Putnam, F. W., and Neurath, H.: J. Biol. Chem. **150**:263, 1943.

33. Kuhn, R., and Bielig, H. J.: Ber. ü. d. deutsch. chem. Gesellsch. **73**:1080, 1940.

34. Baker, Z.; Harrison, R. W., and Miller, B. F.: J. Exper. Med. **74**:621, 1941.

foam cells, or by direct infiltration into the subendothelial space, causing the appearance of stringy or homogeneous small blister-like formations, which secondarily are invaded by endothelial and histiocytic foam cells. Neither the vascular lesions nor the limited foam cell transformations of the reticuloendothelial cells in the adrenal glands, the liver and the lungs gave any indication that desquamated reticuloendothelial cells circulating in the blood become attached to the vascular wall and penetrate into it. The evidence obtained was unequivocally against this contention.

Mention may be made in this connection of the occurrence of medial calcifications in the aortas of several rabbits receiving cholesterol only. In some instances the lesions were seen without atheromatous deposits in the overlying intima. Danisch [35] called attention to similar changes in cholesterolized rabbits, and Hueper [1d] reported such lesions in dogs in which atheromatosis was produced with methylcellulose. These observations provide additional support for the claim that disturbances in the colloidal equilibrium of the plasma lipoids play a causal role in the production of medial calcifications of the Mönckeberg type (Hueper [1a, d]).

Some observations suggest that in certain respects there appears to be an antagonism between the factors favoring atherosclerosis and those active in the production of thrombosis. Healthy blood vessels seem to be coated with a layer of low wettability (Lampert [36]; Hauser, Andreas and Tucker [37]). If the blood vessels become diseased, wettability increases and causes concentration and conglomeration of platelets and later of blood corpuscles at the interface. Atherosclerotic vascular lesions, on the other hand, are relatively rarely associated with thrombosis. The normal antithrombin activity of the serum is accentuated after injections of various macromolecular substances (ovalbumin, gelatin, horse serum, starch, glycogen, methyl cellulose, polyvinyl alcohol), some of which are of nonantigenic type and causally involved in the production of arterial atheromatous lesions (Volkert [38]; Hueper [1c, d]). Bürker [39] noted that all coagulation-inhibiting agents prevent agglutination of platelets, while Hueper, Landsberg and Eskridge [40] recorded a decrease of platelets following injections of polyvinyl alcohol, which readily elicits atheromatous changes.

It is moreover a fact that thrombosis occurs much more frequently in the veins (76.3 per cent) than in the arteries (23.7 per cent). Conversely, atheromatosis is much more prevalent in arteries than in veins. Two factors may account for these differences. The slowness of the venous blood flow favors conglutination of blood cells and wettability of the vascular wall. The adherence of cholesterol to the globulins, on the other hand, prevents deposition of lipoids during their circulation in the vascular tree unless there is instability of the colloidal lipoidal equilibrium causing flocculation of the lipoids, especially at the sites where high turbulence of a swiftly moving blood stream occurs. Inasmuch as the higher carbon dioxide tension of the venous blood favors increased electrical discharge of the negatively charged platelets, conglutination of these elements is enhanced in the venous blood (Stuber and Lang [41]; Klinke [4]). Whether or not differences in oxygen–carbon dioxide tension between the arterial and the venous blood influence significantly the lipoidal colloidal vibratory lability of the arterial blood is uncertain. Finally, it may be mentioned that Pohle and Stewart [42] noted that an increase of the blood fatty acid as seen in lipemia accounts possibly for the abnormally accelerated clotting time observed under these conditions. The intravenous injection of wetting agents causes similarly a marked tendency toward local thrombosis and hemorrhage. From the physicochemical properties of these agents it appears to be likely that suitable surface-active substances of the synthetic type might increase the dispersion and deflocculation of the plasmatic lipoids and thus counteract any abnormal tendency toward precipitation of these constituents, especially of the hydrophobic cholesterol.

SUMMARY

Tergitol Penetrant 08 and Nacconol FSNO given by mouth elicited degenerative and calcifying lesions in the aortas of normal rabbits in addition to causing pulmonary edema, hyperemia and hemorrhages, and pleural effusion. Triton K-60 given by the same route did not produce any vascular reactions.

35. Danisch, F.: Beitr. z. path. Anat. u. z. allg. Path. **79**:333, 1928.

36. Lampert, H.: Die physikalische Seite des Blutgerinnungsproblems und ihre praktische Bedeutung, Leipzig, Georg Thieme, 1931, p. 125.

37. Hauser, E. A.; Andreas, J. M., and Tucker, W. B.: Indust. & Chem. (Indust. Ed.) **31**:32, 1939.

38. Volkert, M.: Biochem. Ztschr. **314**:34, 1943.

39. Bürker, K.: Arch. f. d. ges. Physiol. **102**:36, 1904.

40. Hueper, W. C.; Landsberg, J. W., and Eskridge, L.: J. Pharmacol. & Exper. Therap. **70**:201, 1940.

41. Stuber, B., and Lang, K.: Biochem. Ztschr. **213**: 460, 1929.

42. Pohle, F. J., and Stewart, J. K.: Am. J. M. Sc. **198**:622, 1939.

Similar pulmonary responses were seen in rabbits following repeated intravenous injections of Nacconol NRNO, while rabbits given injections of Triton NE exhibited marked irritation of the intestine. Rabbits into which Nacconol NRNO or Aerosol OT had been injected intravenously showed hyaline degenerated cerebral arteries and renal arterioles. Carcinomatoid proliferations were found in the lungs of rabbits fed Triton K-60 and Nacconol FSNO as well as in a cholesterolized rabbit given injections of Aerosol OT.

Following repeated oral administration of Aerosol OT, Triton NE and lithium iodide plus potassium thiocyanide in rabbits fed daily 0.5 Gm. of cholesterol in oil, it was noted that Aerosol OT seemed to interfere to some degree with the development of hypercholesteremia and atheromatosis, whereas Triton NE and lithium iodide plus potassium thiocyanide appeared to hasten and aggravate these processes.

Some of the evidence suggests that in certain respects there is an antagonism between the factors favoring atherosclerosis and those active in the production of thrombosis related to the wettability of the vascular wall, the circulatory conditions and possibly also the oxygen–carbon dioxide tension of the blood.

EXPERIMENTAL PATHOLOGY AND PHYSIOLOGY OF THE ADRENAL CORTEX

PRODUCTION OF ADDISON'S DISEASE IN LABORATORY ANIMALS

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Since Thomas Addison demonstrated the relation of the adrenal glands to Addison's disease in 1855, many observers have attempted to investigate the function and the pathologic alterations of the adrenal glands by producing experimental Addison's disease in laboratory animals. The experimentation, initiated by Brown-Séquard in 1856 and continued up to the present time, consisted of surgical ablation of the adrenal glands or their destruction by mechanical, thermal, chemical, bacterial or other means. None of these attempts proved successful except that in animals which do not possess accessory adrenal cortical rests complete bilateral adrenalectomy, uncomplicated by important contributing factors, produced symptoms which resembled those occurring in acute crises or in the moribund state of human Addison's disease.

A few years ago the successful production of chronic and subacute, as well as acute, degeneration of the adrenal cortex by experimental procedures was accomplished by me. The pathologic alterations in the adrenal glands were comparable or identical with those which occur in certain types of human Addison's disease. The clinical condition of the experimental animals and the course of the disease were quite similar to what is seen in human beings. These experiments were reported in a preliminary communication.[1] The investigation was interrupted when the department of experimental medicine at Western Reserve University was discontinued. Some of the experiments were conducted at the University of Chicago. The investigation was resumed in the laboratory of experimental endocrinology at the University of Pittsburgh and was extended to include a much larger number of animals and a greater variety of experiments.

From the Laboratory of Experimental Endocrinology, University of Pittsburgh School of Medicine.

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1. Rogoff, J. M.: Proc. Soc. Exper. Biol. & Med. **29**:1240, 1932.

Various degrees of degeneration and necrotic changes were produced in the adrenal glands by subtotal vascular occlusion. Often the experimental procedure induced selective degeneration of the cortex, leaving the medulla intact. A similar process with resulting Addison's disease has been induced accidentally in human beings in consequence of surgical operation on the adrenal glands as a therapeutic measure in the treatment of hypertension, diabetes, hyperthyroidism and various other diseases supposedly related to dysfunction of these glands. Attention has been called to the possible fatal outcome of this practice.[2]

The probability that experimental Addison's disease can be produced by partial but adequate vascular occlusion of the adrenal glands was suggested from the correlation observed between clinical and pathologic observations on Addison's disease and my observations on experimental insufficiency of adrenal cortex. It was supported also by knowledge of the aforementioned accidental results of operation on the adrenal glands in clinical practice. The degeneration of these glands observed at autopsy in some of the clinical cases was similar to that created in the experimental animals.

Among the autopsies in the relatively large series of cases of Addison's disease which were studied was a comparatively great proportion in which the pathologic condition of the adrenal glands was that which has been described as *cytotoxische* (cytotoxic) and as *vaskulare Schrumpfnebenniere* (vascular adrenal atrophy) (Kovacs [3]). In one of these cases [4] there was no evidence of tuberculosis in other organs except a healed focus at the hilus of the left lung. Both adrenal glands had undergone extreme atrophy; a few scattered nodules of cortical tissue, with some medullary cells interspersed, were found on each side. Reliable pathologists classified the changes seen in sections of one of these glands as "cytotoxic" or "idiopathic" atrophy. However, when serial sections of the nodules were made and appropriately stained, careful search revealed the presence of tubercle bacilli, although no evidence of caseation or calcification was observed. Fibrous tissue had replaced

2. Rogoff, J. M.: J. A. M. A. **106**:279, 1936.

3. Kovacs, W.: Beitr. z. path. Anat. u. z. allg. Path. **79**:213, 1928.

4. Rogoff, J. M.: J. A. M. A. **99**:1309, 1932 [case 3].

the degenerated areas, probably inducing vascular occlusion in other zones.

This observation suggests that while the invasion of the adrenal gland by tubercle bacilli did not result in caseous degeneration it might be that a low grade inflammatory process was followed by some necrosis and fibrosis. This, in turn, causing vascular distortion, thrombosis and occlusion, might have induced further degeneration of the gland until degeneration was extensive and regeneration was prevented by inadequate circulation of blood. The foregoing suggestion appears more likely than the assumption of "cytotoxic" degeneration and atrophy of the adrenal glands in Addison's disease. It is in harmony with Kovacs' description of the change as *vaskulare Schrumpfnebenniere*.

If it is accepted that a specific cytotoxin affects the adrenal glands in tuberculosis, the question arises why so prevalent a disease does not lead to a much greater incidence of Addison's disease, tuberculosis being the most common etiologic factor in that disease. In nearly all of the cases of Addison's disease in which so-called cytotoxic atrophy of the adrenal glands was present in this series, Ghon's tubercle could be identified. Indeed, in many instances a roentgenogram of the chest reveals such a primary focus as diagnostic evidence early in the disease. In cases of systemic tuberculosis the adrenal glands are often found to be extensively involved with caseous necrosis, calcification and fibrosis. Nevertheless, this does not necessarily produce Addison's disease, a residual portion of healthy adrenal cortex sufficing to prevent the development of that syndrome.

The volume of blood flowing through the adrenal glands is large. Excepting the thyroid gland, particularly if this gland is hyperplastic, the blood flow of the adrenal gland is greater than that of any other organ of the body. The anatomic and physiologic significance of the collateral venous anastomoses in relation to the adrenal glands was studied by Cow [5] and the relation of the circulation to thrombosis of the adrenal vein by Seecof.[6] The anatomic origin and the distribution of the blood vessels of the adrenal gland favors the large blood flow (Rogoff [7]). In view of this fact it appeared that it should be possible, by appropriate interference with the vascular distribution, to effect a reduction in the circulation of sufficient magnitude to produce subtotal degeneration with consequent gradual incomplete necrosis of the gland. This could be expected to induce physiologic disturbances corresponding to subacute and chronic adrenal insufficiency.

MATERIAL

One hundred and fourteen cats and 54 dogs were used in this investigation. There were variations in the degree of adrenal degeneration and in the severity of the resulting insufficiency. The extent of degeneration and of regeneration or the degree of adrenal insufficiency that will result from subtotal vascular occlusion in the individual animal cannot be predicted in advance. However, the variations that occur in a large series of experimental animals are valuable in that they increase the significance of the observations on the adrenal glands from the point of view of experimental pathology and physiology.

5. Cow, D.: J. Physiol. **48**:443, 1914.

6. Seecof, D. P.: Proc. New York Path. Soc. **26**: 126, 1926.

7. Rogoff, J. M., in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1932, chap. 23.

METHOD

Since in cats and dogs the arterial blood supply of the adrenal gland is derived from small branches reaching the gland from at least three sources, it is not convenient to locate a definite blood vessel for ligation. The ligature, therefore, was adjusted to include some of the venous branches. Usually a ligature was placed around the adrenal vein at its junction with the vena cava (or renal vein) so as to include an adjacent area corresponding to about one third of the surface of the gland. Care must be exercised to avoid inclusion of neighboring nerve structures. In addition, the lumbar end of the lumboadrenal vein was ligated in most cases, sometimes including an adjacent area, especially in dogs.

Fine catgut or silk was used for the ligatures. There is an advantage of catgut in that it is absorbed and does not interfere with cutting sections of the gland for histologic examination. However, the ligations were more successful when silk was employed. The use of silk (or linen) thread for ligatures is advantageous in that it facilitates locating the remains of the gland when the adrenal atrophy and necrosis are extreme.

Various procedures were tried to determine the most satisfactory method of obtaining the desired results. They were as follows:

1. Ligation of vessels of both adrenal glands in one operation.

2. Unilateral ligation followed by contralateral ligation after various intervals.

3. Excision of one adrenal gland and ligation of vessels of the other gland in one or two operations; in some cases the order was reversed.

4. Religation, done when clinical manifestations of adrenal insufficiency failed to develop.

In a number of instances, exploratory laparotomy was performed at different intervals after ligation of adrenal vessels, with the purpose of inspecting the glands. In some cases in which one gland was atrophic or obviously undergoing degeneration while the other appeared to be less affected or unaffected, the latter was excised.

In one series of experiments the adrenal gland was excised one day to some weeks after the vascular ligation, to permit observations on the relation of regeneration to degeneration. The glands were sectioned in different areas, and when marked atrophy was present, serial sections were made for histologic examination.

EXPERIMENTAL RESULTS

The most striking result obtained in the adrenal gland by subtotal vascular occlusion was selective degeneration of the cortex. Sometimes the medulla was also involved but in a large proportion of cases it remained intact (fig. 1). The pathologic changes were found to be the same as those which characterize "cytotoxic" atrophy of the adrenal gland in human Addison's disease.

This result was obtained in a large proportion of the experiments regardless of the procedure followed. It was more commonly produced in a single operation if only one ligated gland remained, the other having been excised. Not only the pathologic changes produced in the adrenal glands but the course of the physiologic disturbances, the changes in the chemical composition of the blood and the clinical manifestations in a majority of the animals all resembled, and in most respects were identical with, those observed in various stages of Addison's disease.

In many of the earlier experiments acute total necrosis of the adrenal glands resulted from attempted subtotal ligation of the blood supply. Either the ligature included too great an area of the vascular distribution or the procedure was followed by inflammatory reaction, formation of scar tissue and occlusion of blood vessels that had not been included in the ligation. Sometimes this was the result of bacterial infection.

Later it was observed that if the ligature at the hilus of the gland included less of the periadrenal vascular area, acute necrosis of the gland could be avoided. Sometimes this was inadequate for obtaining sufficient degeneration of the gland to produce physiologic disturbances. In such cases a second or even a third operation was performed, with ligation of an area adjacent to that of the original ligature. Usually, one or two ligations sufficed to induce anemic necrosis with consequent chronic or subacute adrenal insufficiency, depending on the extent and the speed of degeneration and the degree of regeneration of cortex which ensued.

Some of the animals failed to manifest evidences of adrenal insufficiency in spite of extensive ligation of the blood supply of the glands. In some (cats) this was due to the presence of accessory adrenal cortical bodies. In others it was observed that regeneration of adequate circulation was effected through collateral vascular anastomoses, and thus extensive adrenal degeneration was prevented. In still others it was observed that degenerative changes had occurred which alone could have been responsible for physiologic disturbances but which were exceeded by regeneration of cortex sufficient for functional recovery.

Illustration of only a few of the different types of results obtained will suffice to demonstrate the various groups into which the animals can be divided. The figures and the condensed protocols that are included here represent some typical observations made in the different groups of experimental animals. A division can be made into (*a*) animals with chronic or subacute adrenal insufficiency which ultimately recovered, (*b*) a similar group which ultimately succumbed, (*c*) those in which acute adrenal insufficiency developed and which died in a short time, and (*d*) those in which only slight or no evidences of insufficiency developed and which recovered fully because of inadequate interference with the adrenal circulation or because regeneration was more active than degeneration.

One of the typical experiments demonstrating the principal pathologic changes following subtotal vascular occlusion in the adrenal glands is illustrated in figures 1 to 4. The animal, a cat, survived fifty-one and a half days following bilateral adrenal vascular ligation. A condensed protocol of the experiment follows.

Protocol 1.—In a male cat weighing 3.4 Kg. the left lumboadrenal vein with the adjacent area was ligated at the vena cava and at the lumbar end; the right lumboadrenal vein was treated the same as the left and, in addition, two small anastomotic branches joining the vein above the gland were ligated. The animal survived fifty-one and a half days after the subtotal vascular occlusion.

The cat was in excellent condition for five days; then anorexia and moderate asthenia developed, lasting four or five days, followed by complete recovery. The clinical condition was good, thereafter, with occasional anorexia, until a week to ten days before death, when the anorexia and asthenia increased. Two days before death anorexia was complete, asthenia marked and apathy pronounced; at intervals the cat manifested symptoms referable to the nervous system and emitted cries. On the fifty-first day the animal showed extreme asthenia and irregular cardiac action associated with a semicomatose state; this merged into complete coma, which terminated in death late that evening.

At autopsy there was moderate congestion of the mucosa of the alimentary canal with one ulcer in the stomach, the pancreas showed marked congestion and both adrenal glands were extremely atrophied.

Both glands presented the characteristic histologic picture of so-called cytotoxic adrenal atrophy. The subtotal vascular occlusion had induced selective degeneration of the cortex. The medulla was intact and appeared perfectly normal (figs. 1 to 3). The cortical atrophy was extreme, with extensive degeneration and fibrosis. The normal structural arrangement of the cortical zones was absent, the cortical tissue being replaced largely by fibrous tissue (fig. 2). A few microscopic areas of regenerated and regenerating cortical cells were present, surrounded by fibrous tissue. Here and there were areas in which degeneration and regeneration of cortical tissue were going on simultaneously. These groups of cortical cells could not be identified as belonging to any one of the characteristic zones of a normal adrenal cortex. The cells were irregular in shape, size and arrangement. Some presented normal staining, while in others the cytoplasm was poorly stained and the nuclei were absent or degenerating. Some of the cells had hyperchromatic nuclei, and in a number the nuclei were pyknotic (fig. 1). In some areas there were zones of degeneration showing fatty acid and cholesterol crystal spaces (fig. 4).

Nearly all of the cats and dogs whose adrenal glands presented the pathologic changes of cytotoxic atrophy had shown symptoms of chronic or subacute adrenal insufficiency. The course of these manifestations was, in all of the important

composition of the blood during exacerbations or crises, as will be illustrated farther on, were the same as those usually found in Addison's disease.

This type of adrenal degeneration together with evidence of chronic or subacute adrenal in-

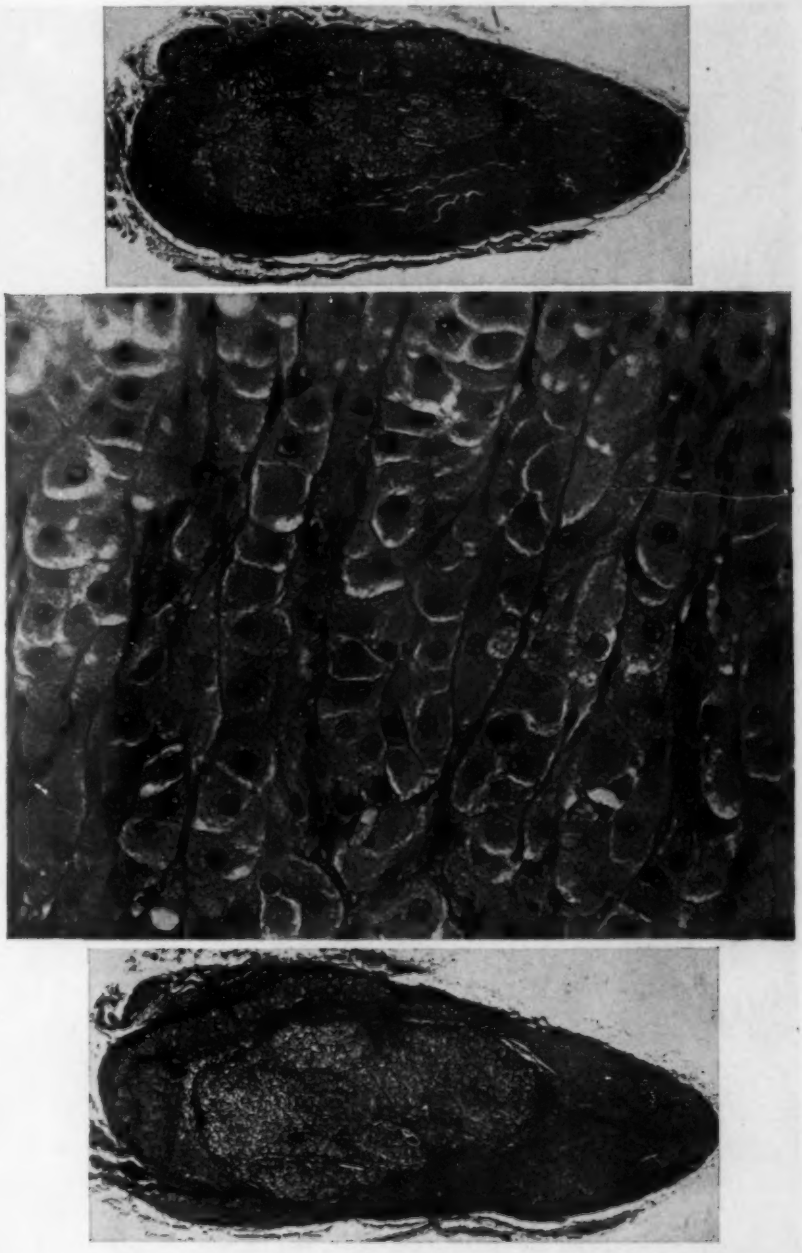


Fig. 1.—At top and bottom are photomicrographs of sections of the right and the left adrenal gland of the cat described in protocol 1 (× 16). At center is a section of the right adrenal gland in an area of regenerated cortex (× 300). The medulla is intact. The cortex is almost completely degenerated; there are small areas of regeneration. The right adrenal gland shows fatty acid and cholesterol crystal spaces in the degenerating zone. Fibrous bands replace areas of preexisting cortex.

indications, comparable or identical with that which is commonly observed clinically in human Addison's disease. Exacerbations and remissions occurred, and the changes in the chemical

sufficiency followed subtotal vascular occlusion in approximately 60 per cent of the animals. In another 25 per cent acute insufficiency developed, and these survived only as long as is usual after

bilateral adrenalectomy. In these animals the ligation was too extensive, and total or almost total necrosis of the adrenal glands resulted. In about 10 per cent of the experimental series ligation was ineffective. The interference with the circulation was inadequate, and only focal degeneration occurred. These animals recovered and survived indefinitely, without symptoms of adrenal insufficiency having developed. About 5 per cent (cats) survived without symptoms because of the presence of accessory adrenal bodies, which were discovered at autopsy.

An important point may be mentioned here in connection with experimental studies of adrenal insufficiency and with the use of adrenalectomized animals for standardizing the potency of preparations of adrenal cortex. It is well known that in certain species, e. g., rats and rabbits, there is a high incidence of accessory adrenal cortical bodies and that adrenalectomy, therefore,

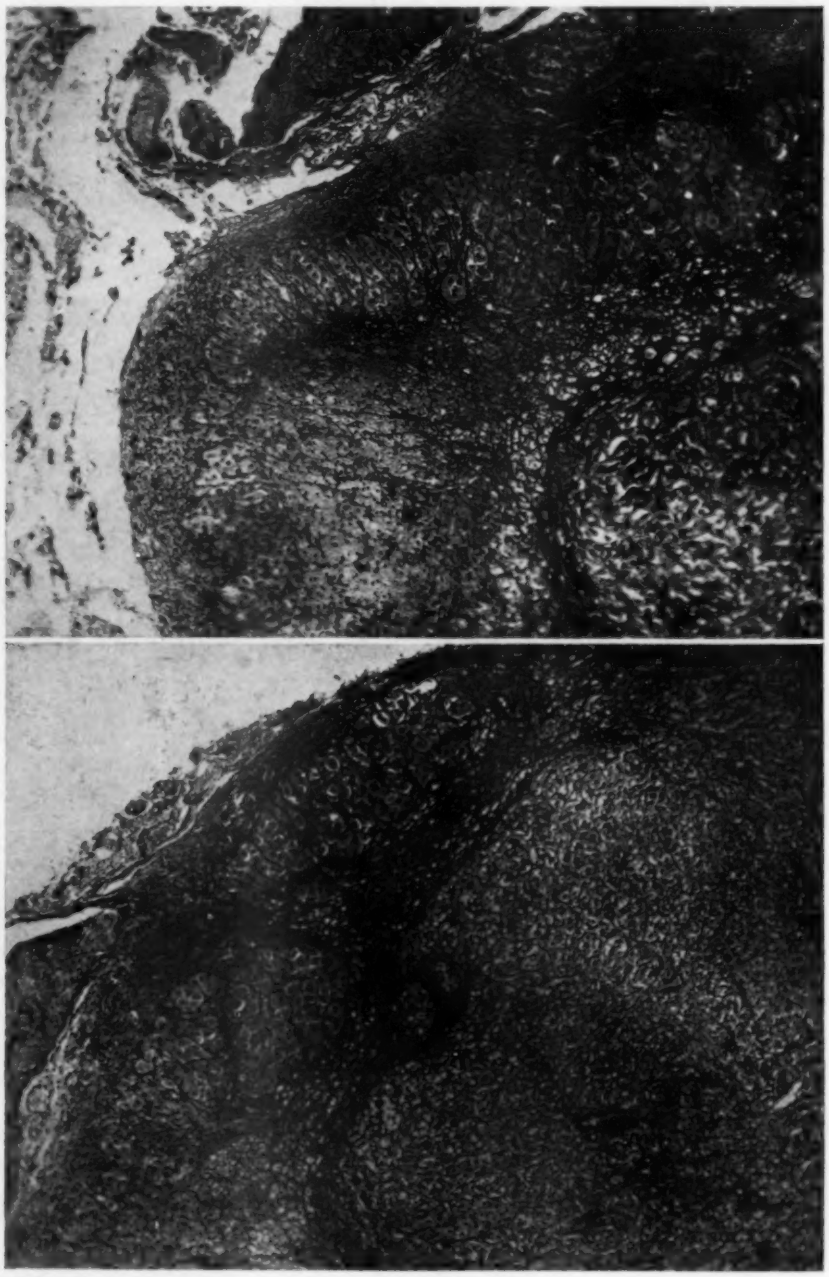


Fig. 2.—Photomicrographs of sections of the left adrenal gland of the same cat as in figure 1: upper, ×83; lower, ×67. Intact medulla is separated by fibrous bands from regenerated cortical areas. Note zones of degenerated and degenerating cortex.

cannot be relied on to produce a desired insufficiency.

In cats the incidence of accessory adrenal bodies has been found to vary from about 15 to 20 per cent. Often these bodies are readily seen on the capsule of the adrenal gland or near the gland and can be excised. However, they may be concealed in other locations and can easily escape detection. An excellent example of this is illustrated by the following condensed protocol of one of the experiments:

Protocol 2.—In a male cat weighing 1.9 Kg. both lumboadrenal veins were ligated at the vena cava and the lumbar end. Eighty-four days later, when the cat's weight had increased to 2.3 Kg., the right adrenal gland was excised, and one hundred and five days thereafter the left lumboadrenal vein was religated at both ends, and two small anastomotic branches joining the lumboadrenal vein above the gland were also ligated. Thirteen days after the last operation, exploratory laparotomy revealed marked atrophy of the remaining (left) adrenal gland and striking congestion of the pancreas. This animal was killed fifty-six days later, while it was in excellent health.

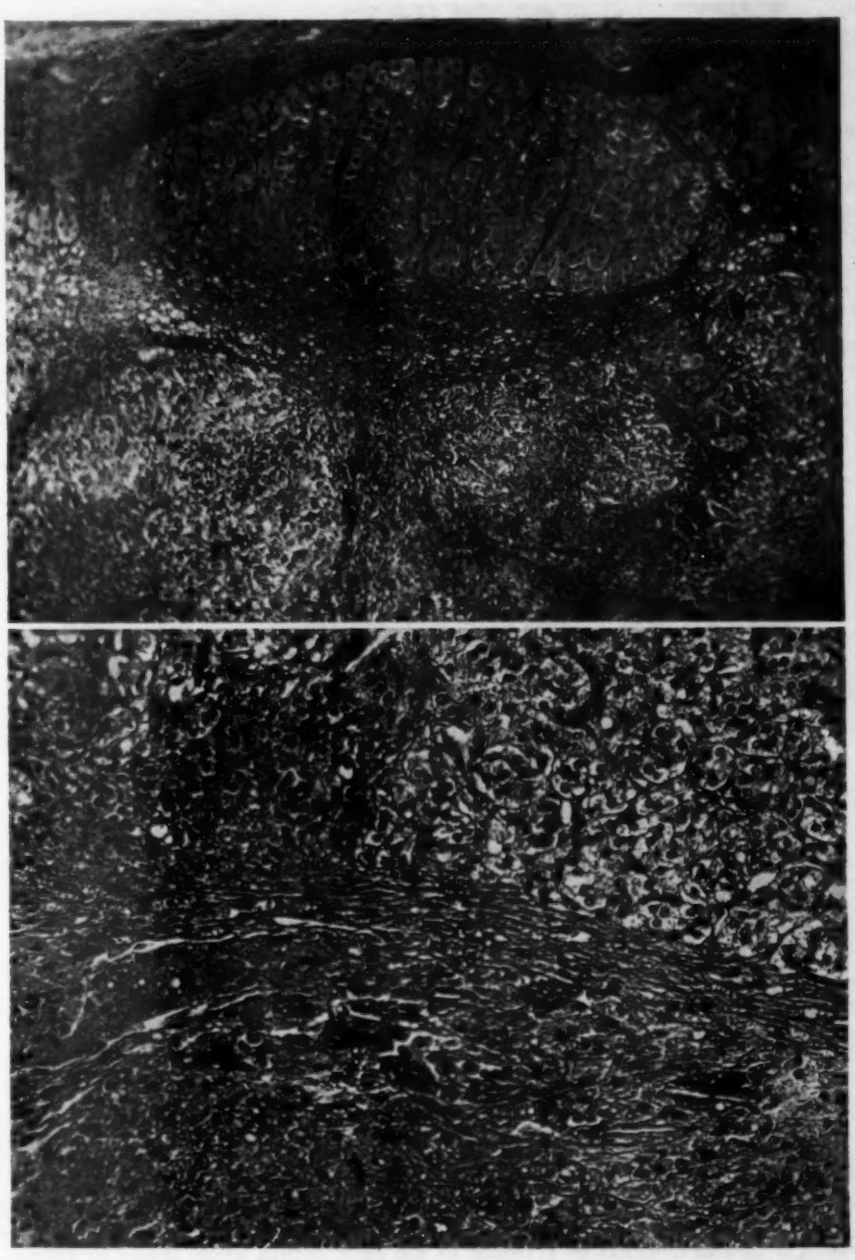


Fig. 3.—Photomicrographs of sections of the right adrenal gland of the same cat as in figure 1: upper, × 68; lower, × 170. The same features as in the left adrenal gland, figure 2, are seen.

Clinical manifestations of adrenal insufficiency were absent during the entire period of survival. The only indication of cortical disturbance observed was the marked congestion of the pancreas noted in the exploratory laparotomy.

The remains of the left adrenal gland consisted of fibrous tissue in which was contained a small area of degenerated and degenerating cortical tissue. The amount of cortical tissue present, in its pathologic state, could not have sustained the life and excellent health of the animal.

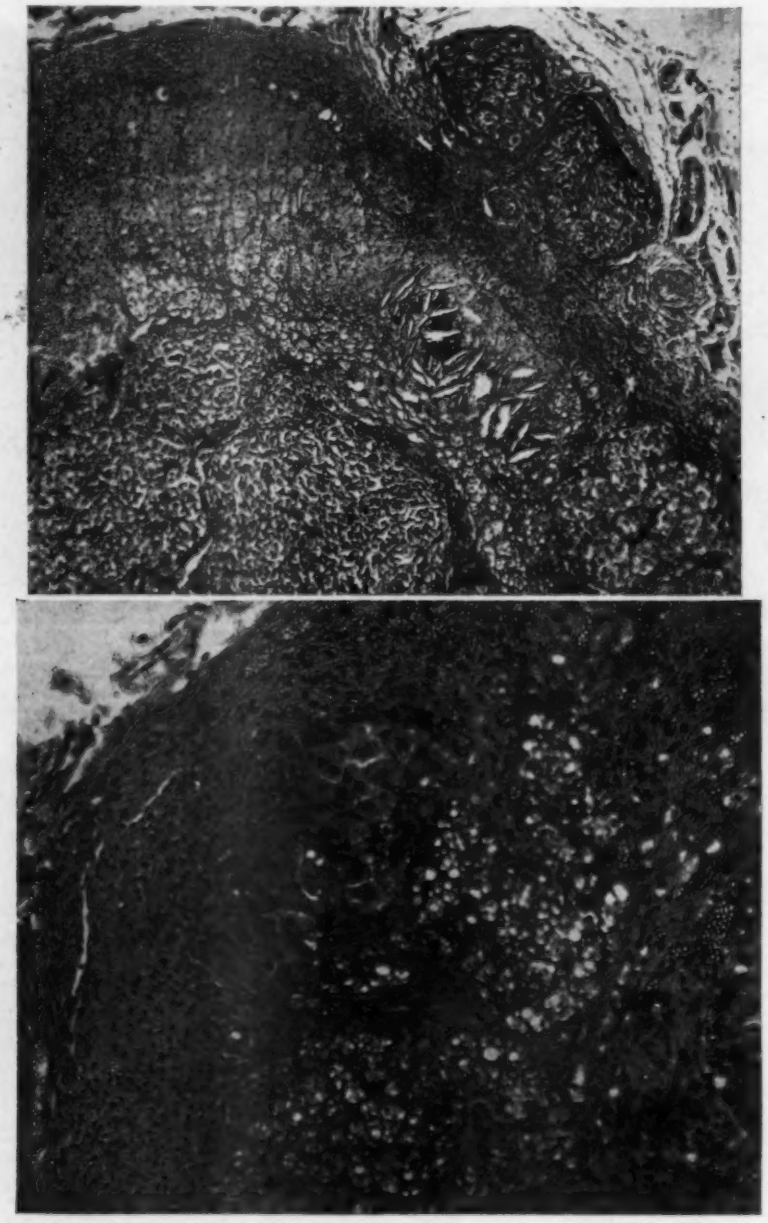


Fig. 4.—At top is a photomicrograph of a section of the right adrenal gland shown at the top of figure 1. Note the fatty-acid and cholesterol crystal spaces, vacuolation and fibrosis in the cortical area, also the regenerated nodule and degeneration in a regenerated area. Below is a portion of normal intact medulla.

Autopsy revealed moderate congestion of the mucosa of the alimentary canal but no gastric ulcers or erosions. The left adrenal gland was represented by only a minute nodule. No accessory adrenal tissue was found grossly.

Histologically, aside from thickening of the capsule and some calcification, the excised right adrenal gland presented no obvious abnormality.

On serial section of the area adjacent to the atrophic remnant of the left adrenal gland, a microscopic nest of cortical cells constituting a well preserved accessory cortical body was seen in close proximity to a nerve ganglion (fig. 5). This microscopic aggregation of cortical cells sufficed to maintain the life and good health of the animal in the absence of normal adrenal glands.

Not all animals having accessory adrenal tissue necessarily survive degeneration or excision of their adrenal glands, even when the accessory bodies are large enough to see with the naked eye. Despite the fact that accessory adrenal tissue is rare in dogs and relatively common in cats, a much greater proportion of cats had acute adrenal insufficiency after vascular occlusion than of dogs. This might be explained by the greater vascularity in the adrenal region and the larger vessels in the dog than in the cat. Furthermore, the ligation probably affects the vascular supply of the accessory as well as of the adrenal gland if both are derived from the same source, so that the accessory gland undergoes necrosis and cannot protect the animal in the aforementioned cases.

It is possible also that while some accessory adrenal rests are capable of functioning, others do not have the capacity to undergo compensatory hypertrophy or otherwise to provide functional compensation. Sometimes it appears that an accessory body can partly supply hormone for bodily requirements but cannot function adequately to meet full functional demand. In such a case, symptoms may be ameliorated for a while and life may be somewhat prolonged, but the inadequate adrenal function finally leads to a fatal outcome.

Symptoms and Pathologic Function.—The symptoms that developed in the various groups of experimental animals corresponded in general with the type and the extent of the cortical degeneration of the adrenal glands following subtotal vascular occlusion. They were the same as those which indicate the physiologic disturbances of Addison's disease and which occur in adrenalectomized animals but were less severe in the cases of subacute and chronic adrenal insufficiency.

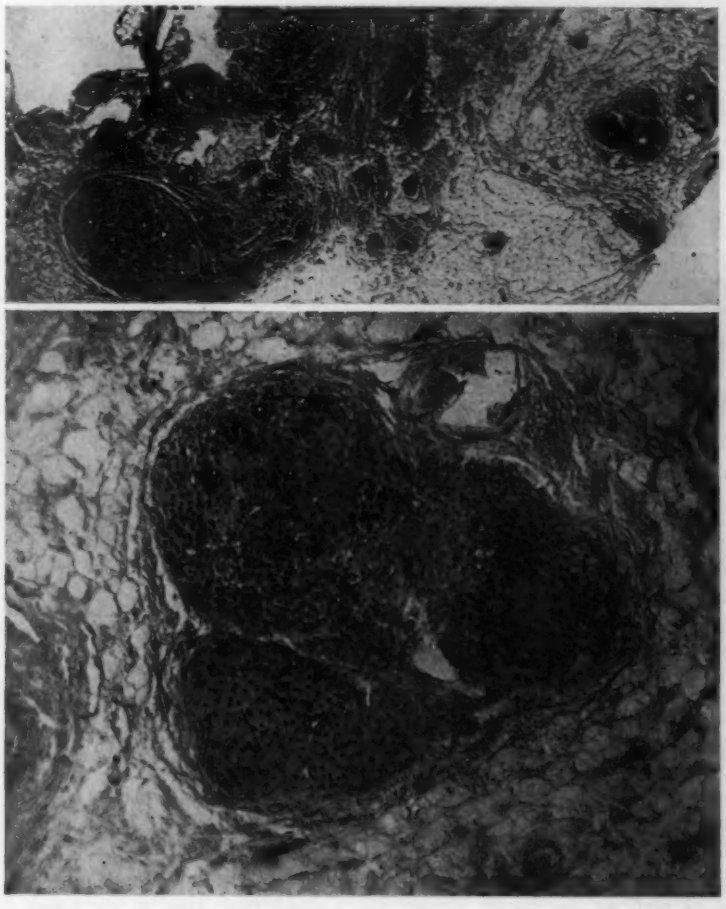


Fig. 5.—The upper photomicrograph shows an accessory cortical rest in close proximity to a nerve ganglion. A high magnification of the cortical accessory body is shown in the lower photomicrograph.

The earliest manifestation was anorexia and aversion to fatty foods. This was sometimes associated with nausea and vomiting (often bile) and other gastrointestinal disturbances, e. g., diarrhea (sometimes bloody). As the degree of adrenal insufficiency became more severe,

other manifestations and greater intensity of those already mentional occurred, including the neuromuscular and circulatory disturbances and derangements of the central nervous system which are commonly seen in adrenalectomized animals. If regeneration of the adrenal cortex supervened, the symptoms subsided and recovery took place.

In addition to the symptoms, the characteristic chemical changes in the blood indicate the pathologic physiology associated with insufficiency of adrenal cortex. These are illustrated in the sample condensed protocols of some of the experimental animals. They indicate a complex metabolic derangement largely manifested by severe intoxication and profound physiologic disturbances, referable to the gastrointestinal, circulatory and nervous systems.

Most of the animals in which the type of adrenal degeneration that resembles cytotoxic atrophy was observed presented the picture of chronic insufficiency. They survived for periods comparable to the period of survival of ineffectively treated patients with chronic Addison's disease. Others in this group in which subacute manifestations developed survived for a shorter period but longer than untreated adrenalectomized animals. These subacute conditions also are comparable with Addison's disease in which the symptoms develop less insidiously and are more rapidly fatal.

Figure 6 illustrates this type of experimental case. Degeneration of the adrenal gland was extremely extensive, leading to necrosis of the entire medulla as well as nearly all of the cortex.

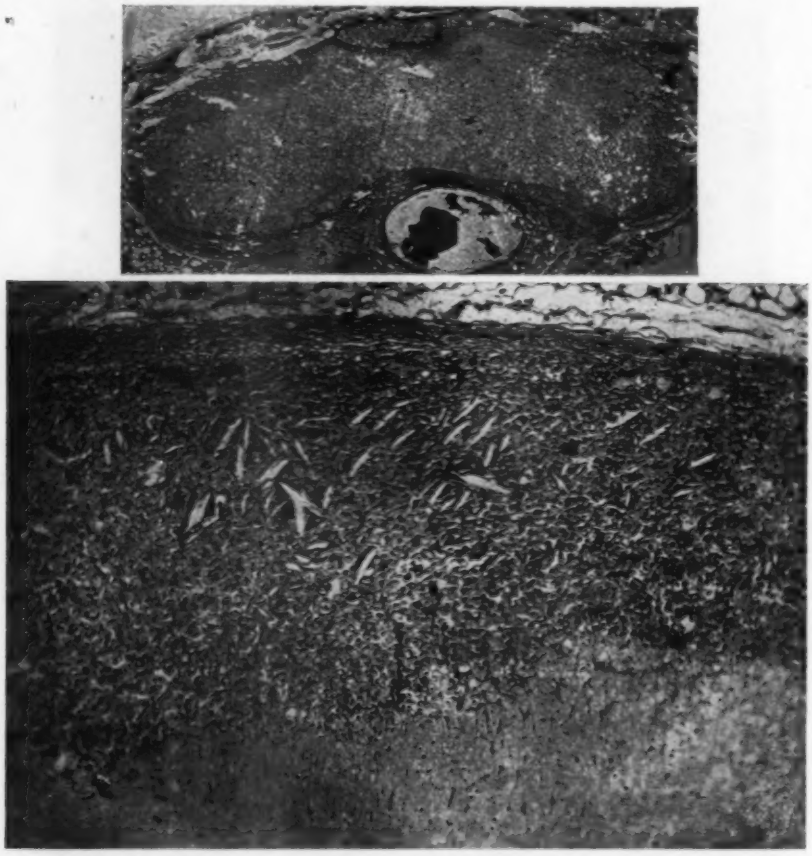


Fig. 6.—Photomicrographs of a section of a cat's adrenal gland showing complete necrosis of the gland, including the medulla as well as the cortex except for a small oval area at the upper border of the cortex representing a regenerated cortical nodule which is undergoing degeneration: upper, × 15; lower, × 90.

Only a small nodule of surviving cortex remained (at the center in the upper part of the circumference of the upper photomicrograph), and that was undergoing rapid degeneration (lower photomicrograph), with scarcely any area of regenerating cells present. This result is illustrated as an example of one of the most severe conditions in this group.

Similar but less extensive degeneration, associated with active regeneration of the adrenal cortex, is illustrated in figure 7. In this instance there was selective degeneration and necrosis of the cortex, the medulla remain-

ing intact. This illustration was selected for demonstration because it represents a group of experimental animals that indicate the possibility of recovery from Addison's disease or from definite cortical insufficiency under favorable conditions. Although degeneration and necrosis of the cortex involve the major portion of the gland, there are sufficient areas of regenerating cortical cells to permit recovery if these zones escape further degeneration. Evidence was obtained indicating that in some cases such favorable conditions may be induced by appropriate administration of a potent extract of adrenal cortex containing the active cortical hormone, interrenalin. If, however, degeneration exceeds regeneration, a fatal outcome is inevitable.

When the vascular ligation results in too extensive interference with blood supply, complete necrosis of the adrenal gland results, involving both the cortex and the medulla. An example of this is illustrated in figure 8 (top). Under these conditions acute adrenal insufficiency develops and the animal dies within the same period as the one which has undergone bilateral adrenalectomy.

Observations in an experiment in which infection occurred at the time of ligation of one of the adrenal glands led to attempts to introduce non-specific infection at the time of ligation of the adrenal blood vessels in a few instances. This was done by passing the ligature across the edge of the operating board. In most cases this resulted in acute total necrosis of the entire adrenal gland with various degrees of purulent infiltration (fig. 8, bottom). In some instances there was abscess formation. The animals succumbed to acute adrenal insufficiency just as did those with excessive vascular occlusion, previously mentioned. The histologic appearance of sec-

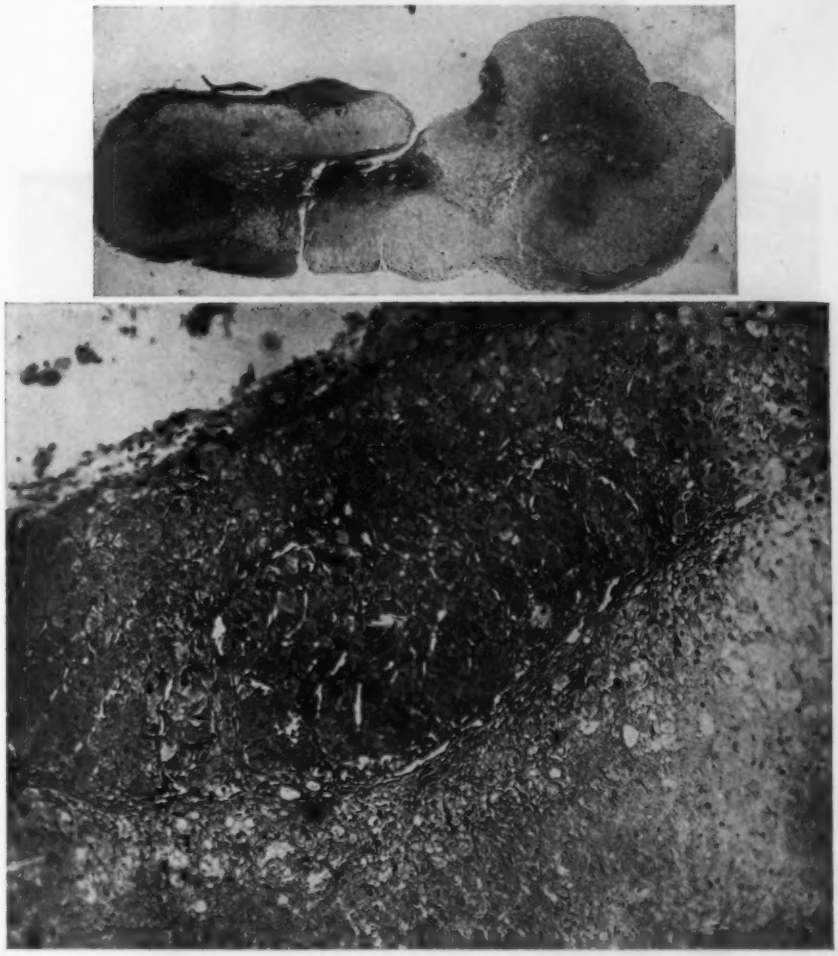


Fig. 7.—At top is a photomicrograph of a section of a dog's adrenal gland (× 8). The medulla is intact; most of the cortex is degenerated and necrotic except small peripheral areas of regenerated and regenerating cortex. Below is a high magnification (× 110) of regenerated cortical area.

tions of the glands from these animals is perhaps comparable with that of sections of the adrenal gland with caseous degeneration and necrosis in Addison's disease.

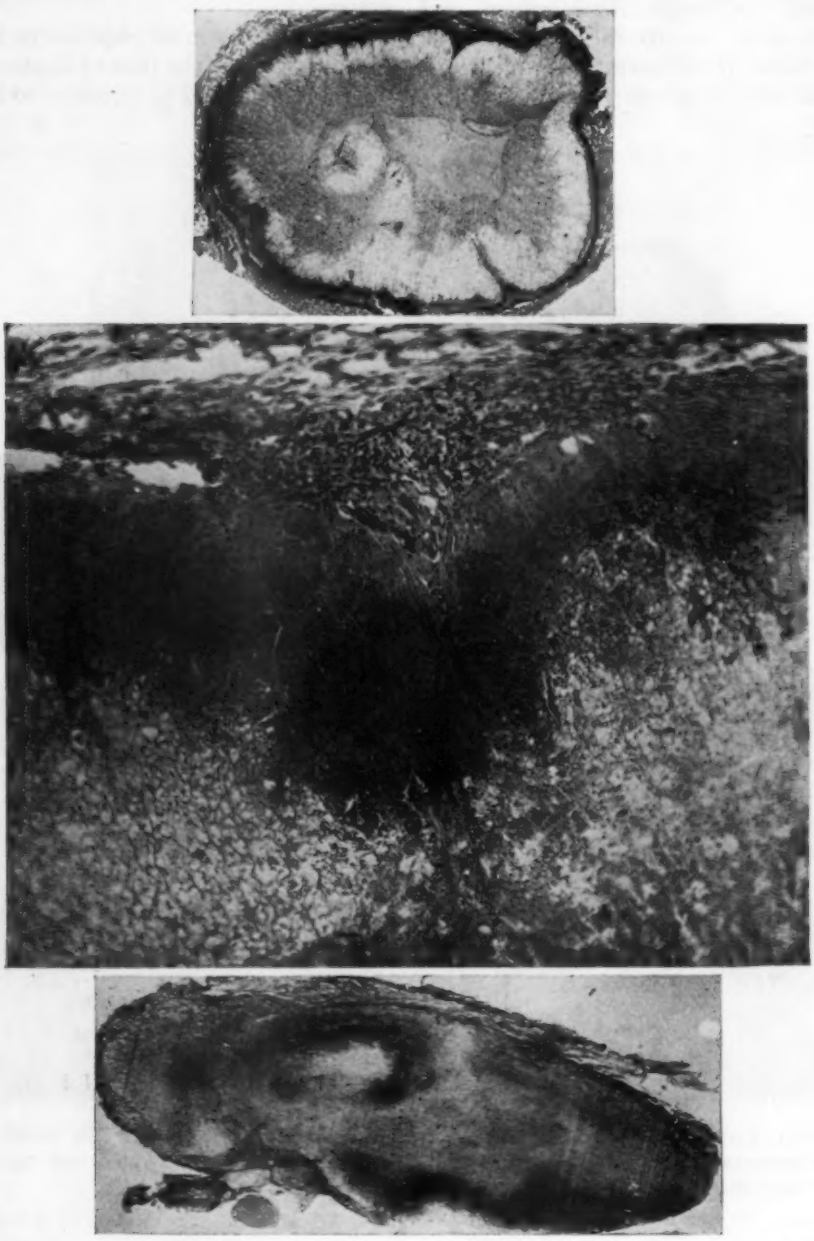


Fig. 8.—At top is a photomicrograph of a section of a cat's adrenal gland showing complete necrosis of the entire gland, including the cortex and the medulla (× 9). At center is a high magnification (× 85) of a degenerating and necrotic portion of the same. At bottom is a section of a cat's adrenal gland with purulent necrosis of the entire cortex and medulla (× 9).

Inadequate vascular occlusion sometimes occurs with ligation. In such a case a relatively large portion of adrenal cortex may escape degeneration (fig. 9). The animal recovers from the operation, and symptoms of adrenal insufficiency fail to develop. Often another operation with application of another ligature is followed by degeneration of the remaining normal cortex, leading to one of the types of degeneration and the clinical manifestations described in the foregoing pages. If both adrenal glands are ligated and one undergoes extensive degeneration and the other only slight degeneration or none, symptoms of adrenal insufficiency may be very slight or may be absent. But when the latter gland is excised, leaving the one that is affected by the

vascular occlusion, chronic or subacute insufficiency develops. An example of this is illustrated by the following condensed protocol:

Protocol 3.—In a male cat weighing 3.66 Kg. The left lumboadrenal vein was ligated at the vena cava. One month later, when the animal weighed 3.7 Kg., the right lumboadrenal vein was ligated at the vena cava and at the lumbar end, also two small anastomotic branches joining the lumboadrenal vein above the gland. Fifty-five days after the second ligation, when the cat weighed 3.7 Kg., the left adrenal gland was excised. The animal survived fifty-two days after the last operation.

No clinical evidence of adrenal insufficiency appeared until about three weeks after the excision of the left adrenal gland (which was unaffected by the venous occlusion). Within the next four weeks there were two periods of anorexia and asthenia, with remissions. The first period lasted about two weeks with an occasional day or two of improvement and final complete clinical recovery for about a week; anorexia again developed, increasing gradually and becoming associated with periods of apathy and asthenia. Two days preceding death anorexia was complete, asthenia marked, and apathy merged into coma terminating in death fifty-two days after excision of the left adrenal gland.

Autopsy showed moderate congestion of the mucosa of the alimentary canal and numerous erosions and ulcers of the stomach, marked congestion of the pancreas, and extreme atrophy of the right adrenal gland. The parathyroid glands were prominent and somewhat larger than normal.

Histologically, the left adrenal gland appeared normal aside from recent traumatic hemorrhage. The right adrenal gland was almost entirely necrotic and fibrous. In one area sections showed necrosis and marked fibrosis of cortex with zones of regeneration in which many cells were undergoing degeneration and others were already degenerated, as is seen in so-called "cytotoxic adrenal atrophy." In general, the histologic appearance was the same as described in protocol 1 and illustrated in figures 1 to 4.

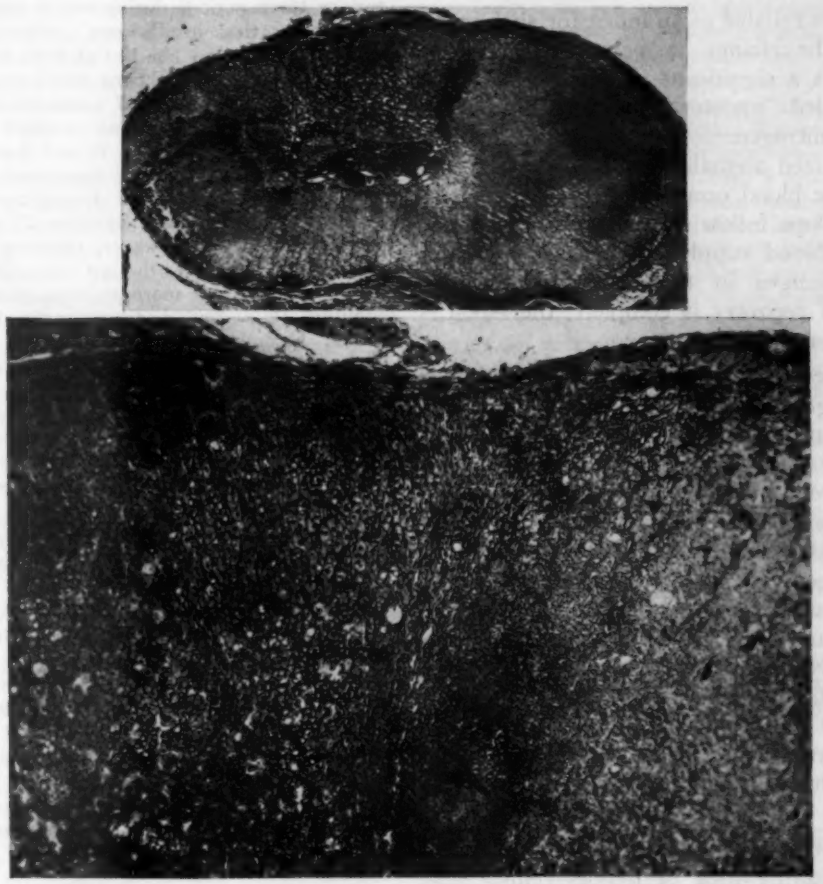


Fig. 9.—At top is a photomicrograph of a section of a cat's adrenal gland (× 11) showing that about one fifth of the cortex escaped the degeneration and necrosis which the balance of the cortex underwent; the medulla is intact. Below is a section showing an area at the junction of normal and degenerated cortex (× 85).

Chemical Observations on the Blood.—In a number of cases observations were made on the total nonprotein nitrogen, urea nitrogen and dextrose levels in the blood. In some cases the level of sodium chloride and that of total serum calcium also were determined. Diminution of sodium chloride and elevation of serum calcium were found in some cases during manifestations

of subacute or acute adrenal insufficiency. But, as demonstrated long ago (Rogoff and Stewart [8]), while reduction of sodium chloride often occurs in adrenal insufficiency, it is not necessarily a constant phenomenon. Normal levels of sodium chloride have been observed in the blood during acute crises in adrenalectomized animals and in patients with Addison's disease. Reduction of sodium chloride, therefore, is not a reliable pathognomonic index for adrenal cortical insufficiency.

Much more reliable as an index for such insufficiency is the change in nonprotein nitrogen which reflects a significant increase in the level of the so-called "undetermined fraction" of the nonprotein nitrogen. As in the studies on adrenalectomized animals, it was found that this change in the blood occurs when adrenal insufficiency develops following subtotal occlusion of the adrenal blood supply. When recovery occurs, the changes in the blood become less marked or, if recovery is complete, the blood is found to be normal.

None of the other components of the total nonprotein nitrogen except the urea nitrogen undergoes significant changes; any changes that occur are too small to account for the increase in the total nonprotein nitrogen. Even the relatively large increase in the urea nitrogen is insufficient to account for the total increase in nonprotein nitrogen. In fact, there is usually a significant increase in total nonprotein nitrogen, reflecting the increased "undetermined" nitrogen, before the level of urea nitrogen begins to rise; as the urea nitrogen increases, the total is augmented more rapidly than the urea nitrogen, yielding a greater increase in the undetermined fraction of nitrogen as the cortical insufficiency increases.

Further evidence was obtained, also, indicating that the prevailing idea of hypoglycemia as a characteristic of adrenal insufficiency is not substantial. This confirms the previous results, obtained in observations on adrenalectomized dogs, in which it was noted that the blood sugar in adrenal insufficiency usually is at about the lower level of the normal range but not at a hypoglycemic level. Sample condensed protocols are given to illustrate the chemical changes in the blood in relation to the clinical manifestations in some of the animals with chronic and subacute adrenal insufficiency.

Protocol 4.—In a male cat weighing 1.9 Kg. the lumboadrenal veins of both glands were ligated at the vena cava and lumbar ends. Approximately eight and a half months later, when the animal's weight was 2.7 Kg., the veins were religated. The cat survived a little more than four months after the second ligation. Its weight at death was 1.1 Kg.

During about four months following the first operation there were occasional periods of anorexia and recovery; then a subacute condition developed, lasting nearly four weeks. During this period there were anorexia, occasional vomiting, apathy and periods of asthenia. Complete recovery followed. Exploration at the time of religation of the adrenal vessels revealed atrophy of both glands to about half of their normal size. The pancreas was markedly congested. In the two months after the second operation there were two or three periods during which symptoms indicated subacute cortical insufficiency, followed by apparently complete recovery. The last of these exacerbations was present about a week, during which there were increasing asthenia, anorexia and somnolence. At this time an examination of the blood revealed total nonprotein nitrogen 106, urea nitrogen 78 and dextrose 70 mg. per hundred cubic centimeters. Improvement followed and fifteen days later the blood showed nonprotein nitrogen 56, urea nitrogen 41 and dextrose 75 mg. per hundred cubic centimeters. However, recovery was incomplete, and about a week after the last examination of the blood the anorexia became more pronounced and constant, and vomiting (bile) and diarrhea occurred frequently, the animal becoming decidedly asthenic. About ten to twelve days preceding death there was a period of spontaneous recovery lasting nearly a week when appetite returned and the cat seemed much stronger and appeared as if it might recover. Nevertheless, an exacerbation developed, with total anorexia, bilious vomiting, marked asthenia, somnolence, convulsions and coma, resulting in death.

Autopsy showed the parathyroid glands enlarged, the heart small (about half normal size) the pancreas markedly congested and both adrenal glands atrophic and sclerotic.

Histologic examination disclosed extreme atrophy of both adrenal glands. Sections of the right adrenal gland showed marked fibrosis. There were degeneration and some regeneration in one larger nodule of cortex, which was extremely vacuolated; there were a few small nests of regenerated cortical cells, some undergoing degeneration. The medulla was spared but was distorted and small.

The left adrenal gland was less fibrous than the right; there was an atrophic area of what appeared to be original cortex, undergoing degeneration, as well as a few small nests of regenerated cortical cells. The medulla was intact.

Protocol 5.—In a male dog weighing 9.9 Kg. the right adrenal gland was excised. Five and a half weeks later, when the weight of the animal was 10.3 Kg., the left lumboadrenal vein was ligated at the vena cava and lumbar ends, including small anastomotic branches. Ten months thereafter, exploratory laparotomy was performed for inspection of the adrenal gland. Death occurred six days later.

The clinical condition was excellent for three months; then there was a period of anorexia and vomiting during a week, followed by muscular asthenia lasting four or five days. The dog recovered and was in good health until bloody diarrhea occurred, about a month later, lasting for a few days, with apparently complete recovery. Another similar exacerbation occurred four months afterward, and yet another a little more than two months later, with remission of symptoms between them. At the time of the last exacerbation, i. e., ten months after the ligation, exploratory laparotomy was

8. Rogoff, J. M., and Stewart, G. N.: Am. J. Physiol. **78**:711, 1926 [table 1].

performed for inspection of the adrenal gland during a period of subacute manifestations of cortical insufficiency. The gland appeared degenerated and atrophic. Eight days before the laparotomy an examination of blood showed total nonprotein nitrogen 59, urea nitrogen 41 and dextrose 70 mg. per hundred cubic centimeters; on the day preceding the operation the nonprotein nitrogen was 63, the urea nitrogen 35 and the dextrose 74 mg. Following the laparotomy, the symptoms increased in severity, and five days after the operation the blood showed nonprotein nitrogen 91, urea nitrogen 62 and dextrose 70 mg. per hundred cubic centimeters. Death ensued on the following day.

At autopsy, aside from the atrophy and degeneration of the adrenal gland the only significant abnormalities were moderate enlargement of the parathyroid glands and congestion of the pancreas. There were lymphoid hyperplasia and enlargement of many mesenteric lymph nodes. One ulcer was noted in the pyloric end of the duodenum.

Histologic sections of the atrophic left adrenal gland showed extreme necrosis of the cortex. The normal structure of the gland was destroyed. Vacuolation was extensive, and large fibrous bands replaced the original cortex in many areas. There were numerous small areas of calcification and some of round cell infiltration throughout the cortex. A few small nests of regenerated cortical cells were present in one area and one larger zone in which degeneration was present. The medulla was intact.

Protocol 6.—In a male dog weighing 8.0 Kg. both lumboadrenal veins were ligated at the vena cava and lumbar ends. About eight and a half months later, when the animal weighed 8.2 Kg., the right lumboadrenal vein was religated as before. The dog survived thirty-eight days after the second operation.

The dog was in excellent condition for about three months after the first operation. During the next ten weeks there were four periods, lasting three or four days each, of anorexia, bilious vomiting and diarrhea (blood), with apparently complete recovery following each exacerbation. After religation of the right lumboadrenal vein the animal's condition was good for thirty-four days. Then an acute crisis developed; anorexia, apathy, bilious vomiting and bloody diarrhea developed, and within three days there was spasticity of muscles, coma and convulsions. At this time examination of the blood revealed total nonprotein nitrogen 225, urea nitrogen 155 and dextrose 210 mg. per hundred cubic centimeters. Death ensued within the day of the examination of the blood.

Autopsy showed marked congestion and hemorrhages in the mucosa of the alimentary canal, numerous ulcers and erosions of the stomach, and not much congestion of the pancreas. The parathyroid glands were large.

Histologically, the normal structure of the left adrenal gland was fairly well preserved. The gland was sclerotic. There was parenchymatous degeneration; numerous foci of degenerated and degenerating cells were scattered throughout the gland, and some areas of regenerating or normal cells.

The right adrenal gland showed extensive, practically complete, parenchymatous degeneration and hyaline necrosis with some fibrosis. The normal structure was destroyed; there were small foci of degenerating cortical cells; almost no adrenal tissue could be recognized.

Protocol 7.—In a male dog weighing 10.5 Kg. the lumboadrenal veins were ligated at the vena cava and lumbar ends. Eight and a half months later, when the animal's weight was 14.2 Kg., the right lumboadrenal vein was religated, and two months thereafter, when the animal's weight was 13.9 Kg., the left lumboadrenal vein was religated. Thirteen months later, when the dog weighed 12.1 Kg., the left lumboadrenal vein was again ligated, but owing to extensive, well organized, firm adhesions from previous operations, the adrenal gland and vein were exposed with difficulty, resulting in traumatic shock. Death ensued the following day.

Clinically the condition of the dog was excellent for about two and a half months; then three exacerbations occurred within approximately eight weeks, with anorexia, bilious vomiting, diarrhea and some apathy and inactivity. Each spell lasted two or three days and recovery followed. At the end of the last of these exacerbations, an examination of the blood showed total nonprotein nitrogen 88, urea nitrogen 52, and dextrose 74 mg. per hundred cubic centimeters.

Clinical recovery was complete, and the animal continued in excellent health up to and after the religation of the right lumboadrenal vein. Following the religation of the vein on the left side, the condition was excellent for eighteen days; then a two to three day period of vomiting and diarrhea occurred, with complete recovery lasting until about eight months later, when another such spell occurred and recovery followed. Between these two exacerbations the blood on two occasions showed (1) nonprotein nitrogen 33, urea nitrogen 16 and dextrose 78 mg., and (2) nonprotein nitrogen 36, urea nitrogen 17 and dextrose 92 mg., per hundred cubic centimeters. At the time of these examinations of the blood the clinical condition of the animal was good.

At autopsy the only significant observations were moderately enlarged parathyroid glands and a strikingly congested pancreas.

Histologic sections of the right adrenal gland showed the structure of the gland distorted. There were extensive vacuolation and degeneration in the glomerular zone but a fair amount of normal tissue in the fascicular and reticular zones, in which there was some degeneration. The medulla was intact.

The left adrenal gland showed recent parenchymatous degeneration and necrosis of the entire gland, including the medulla. No intact cortical cells could be recognized. The fibrous capsule was quite thick but otherwise fibrosis of the gland was not present. There was some recent hemorrhage, and areas of round cell infiltration were present.

Protocol 8.—In a male dog weighing 10.0 Kg. the left adrenal gland was excised, and thirty-six days later, when the animal weighed 10.8 Kg., the right lumboadrenal vein was ligated at the vena cava and lumbar ends, including two anastomotic branches. A year thereafter the right lumboadrenal vein was religated, the adhesions were separated and the gland was left attached to the vena cava by only a small vascular pedicle. Death occurred on the fourteenth day following the last operation.

The dog's condition was excellent until four months after the ligation of the right lumboadrenal vein, when vomiting occurred, but recovery was complete in two or three days. Six weeks later there was a three day period of anorexia, vomiting, diarrhea, apathy and some asthenia. At the end of this period the blood showed total nonprotein nitrogen 58, urea nitrogen 37 and dextrose 80 mg. per hundred cubic centimeters. Recovery followed. Five months thereafter there was a vomiting spell lasting a day or two, with no other symptoms. One week later the blood showed nonprotein nitrogen 46, urea nitrogen 25 and dextrose 83 mg. per hundred cubic centimeters, and a week afterward, on the day preceding the second ligation of the

right lumboadrenal vein, it showed nonprotein nitrogen 41, urea nitrogen 23 and dextrose 90 mg. Five days after the operation the blood showed nonprotein nitrogen 36, urea nitrogen 24 and dextrose 82 mg. On the following day the dog vomited, with considerable bile, and this continued the next day with the onset of acute manifestations, including total anorexia, apathy and asthenia; the blood showed nonprotein nitrogen 113, urea nitrogen 68 and dextrose 85 mg. Two days later the animal lapsed into coma and died late in the evening.

Autopsy disclosed extreme congestion and hemorrhage in the mucosa of the alimentary canal and numerous erosions and ulcers of the stomach, a strikingly congested pancreas and prominent parathyroid glands.

Histologic sections of the right adrenal gland revealed extensive recent necrosis in both the cortex and the medulla; some normal medulla was present, also a small area of apparently normal cortex in which degeneration was going on, as evidenced by many fatty acid and cholesterol crystal spaces. There was extensive fibrosis throughout the gland, replacing cortical areas. The glomerular zone had undergone extensive degeneration and necrosis; the other zones showed extensive degeneration and necrosis, with an occasional area in which a few cells were regenerating and others degenerating.

Protocol 9.—In a male dog weighing 12.9 Kg. the left adrenal vein was ligated at the lumbar and vena cava ends; branches at the anterior and posterior ends of the gland were ligated, including adjacent areas corresponding to about one third of the circumference at each end. Two months later a similar operation was performed on the right side. The dog made an excellent postoperative recovery. Seventeen days after the last operation subacute symptoms of adrenal insufficiency developed, vomiting (bile), anorexia, apathy and some asthenia, lasting twelve days; the symptoms increased in severity for about a week and then gradually subsided until recovery was complete. On the sixth day of this period, when the symptoms were definite, an examination of the blood revealed total nonprotein nitrogen 296, urea nitrogen 182 and dextrose 87 mg. per hundred cubic centimeters; the same specimen when examined the next day in another laboratory showed nonprotein nitrogen 277, urea nitrogen 169 and creatinine 14.6 mg.

Following recovery from this condition, the animal was in excellent clinical condition for about four months, during which there were occasional periods of a day or two of anorexia, sometimes with vomiting; during one of these periods the blood showed nonprotein nitrogen 63, urea nitrogen 45 and dextrose 94 mg., and a week later, nonprotein nitrogen 46, urea nitrogen 28 and dextrose 85 mg., per hundred cubic centimeters. Thereafter the animal's condition was excellent; four examinations of the blood during the period of apparently good health gave results as follows: nonprotein nitrogen 42, 47, 37 and 42 mg.; urea nitrogen 28, 23, 28 and 22 mg.; dextrose 75, 93, 83 and 93 mg. An exploratory laparotomy during this period revealed atrophy of the right adrenal gland and discoloration (degeneration) of its lower pole. The left adrenal gland showed little if anything to indicate degeneration; it was surrounded by adhesions.

Fifteen months after ligation of the right adrenal vein a second exploratory laparotomy revealed considerable atrophy of the right adrenal gland and no apparent change in the left; the left adrenal vein was religated in the same manner as was the right vein originally. The postoperative recovery was excellent, and the blood three days after the operation showed nonprotein nitrogen 43, urea nitrogen 18 and dextrose 81 mg. per hundred cubic centimeters. Two weeks later, the animal being in excellent condition, the figures were nonprotein nitrogen 36, urea nitrogen 18 and dextrose 97 mg. The animal continued in excellent health with an occasional period of a day or two in which there was anorexia and sometimes vomiting or apathy.

Approximately eight months after the second laparotomy, another exploratory operation was performed. The left adrenal gland had undergone extreme atrophy, and adrenal tissue could not be identified near the ligatures; there were firm adhesions and a small nodule, about 4 or 5 mm. in diameter, near the celiac ganglion. One month later, another operation revealed the same abnormal conditions on the left side and two small nodular masses of recognizable adrenal cortical tissue, about 3 and 4 mm. in diameter, as the remains of the right adrenal gland; these apparently were capable of adequate function since the animal continued in excellent health after the operation.

About two months after the fourth laparotomy, it was decided to determine whether the nodule on the left side contained enough adrenal tissue to sustain life; the remains of the right adrenal gland were excised; acute adrenal insufficiency developed, and the animal died on the sixth day after the operation.

At autopsy the mucosa of the alimentary canal was congested, and there were two small ulcers of the stomach. The pancreas was moderately congested. The parathyroid glands were prominent and decidedly larger than normal. Only a minute nodule was found near the ligature at the site of the left adrenal gland.

Sections of the nodule which was found on the left side showed a nerve ganglion and a lymph node bound together by fibrous tissue and surrounded by the fibrous tissue of the adhesions. No adrenal cells could be found.

Sections of the nodules of remaining adrenal gland on the right side, which were excised at the last operation, showed extreme atrophy. The normal structure of the gland was absent. The cortical region was distorted and largely replaced by wide bands of fibrous tissue which coursed in all directions. Between the bands there were areas of cortical cells, varying in extent, showing marked vacuolation, degenerated and degenerating cells, and some regenerating cells, as described in protocol 1. These areas appeared to be original cortex. Here and there were small zones of regenerated cortical masses, surrounded by fibrous bands. The medulla was intact, showing no evidence of degeneration.

Excision of Gland in Which Vessels Had Been Ligated.—In a number of instances observations were made to correlate the clinical symptoms and the chemical changes in the blood with the pathologic manifestations in the adrenal glands. Thus, glands were removed from animals for histologic examination at various intervals, ranging from one day to many weeks, after ligation of the blood vessels. In some cases the glands were removed during a period of subacute or acute adrenal insufficiency, as indicated by the blood changes and other evidences; in other cases the glands were excised shortly after recovery from insufficiency. In many of these cases, of course, the animals were killed.

From these observations it was possible to associate marked blood changes and other significant symptoms with active degeneration of the adrenal cortex. Crises usually were related to extensive degeneration and little if any regeneration; recovery or remissions were associated with mild degrees of degeneration succeeded by active regeneration. Evidence of regeneration concomitant with active degeneration of the adrenal cortex was observed in some cases occurring as early as one or two days after ligation of the adrenal blood vessels; this was observed in no cases, however, of rapid acute total necrosis with or without purulent infiltration of the gland.

Excision of one adrenal gland when both glands have undergone degeneration and atrophy in consequence of bilateral subtotal vascular occlusion may determine the course of events regarding the remaining gland. In some experiments it appeared that degeneration was favored, probably by the suddenly increased functional demand, and the animal declined, succumbing to subacute or acute adrenal insufficiency. The suggestion of this possibility is permitted by the observation that atrophic glands which had existed for a long time without symptoms, because of adequate function of the opposite gland, usually did not show active and recent degeneration until the other gland was excised or caused to undergo more extensive degeneration by religation.

On the other hand, sometimes it appeared that excision of one of the two atrophic glands had stimulated regenerative processes and produced some hypertrophy in the nodules of regenerated cortical tissue as a sort of compensatory mechanism. Incidentally, there was some evidence that this could be accomplished by appropriate administration of interrenalin. It seemed, also, that it was more difficult to induce further degeneration by religation in such a gland than in a gland that had not undergone this apparent hypertrophy.

In a relatively large proportion of the glands in which extensive general or focal necrosis was induced by anemic infarction following the vascular occlusion, the cortex contained numerous foci of calcification. Often these occurred in areas where a large number of fatty acid and cholesterol crystal spaces were present. Sometimes it appeared that a number of these spaces coalesced and were replaced by the calcium deposit, and nearby there were isolated spaces, some being partly filled with the calcium salts. It should be mentioned that foci of spontaneous calcification are not infrequently noted in the adrenal glands of old cats.

COMMENT

The demonstration that completely adrenalectomized animals can be maintained in good health by administration of the adrenal cortical hormone, interrenalin, in the form of a potent adrenal extract (Rogoff and Stewart [9]) led to studies on chronic and subacute adrenal insufficiency induced by inadequate treatment of such animals. This is not identical, nor in many respects comparable, with the physiologic and pathologic alterations which occur in Addison's disease in consequence of chronic degeneration of the adrenal glands.

Successful experimental production of pathologic changes that are similar or identical with those which occur in the adrenal glands in Addison's disease establishes a method for investigating adrenal insufficiency and for making observations on adrenal cortical function. The fact that subtotal vascular occlusion can induce selective cortical degeneration without involving the medulla in a large proportion of cases and that only when the cortical involvement is great enough to result in inadequate function do the manifestations of adrenal insufficiency occur furnishes final proof that Addison's disease is related entirely to the cortex and not to the medulla of the adrenal gland. It also adds substantial evidence for indispensability of cortical and nonindispensability of medullary functions.

If suitable means can be found to induce active regeneration or hypertrophy of cortical cells in atrophic adrenal glands or to control degeneration, the problem of prevention of the development of fatal adrenal insufficiency will be solved. Indeed, clinical observations on the diagnosis and the treatment of Addison's disease (Rogoff [10]) indicate that the administration of a potent extract of adrenal cortex may do this, at least in part. Further investigations, leading to earlier recognition of the onset of adrenal insufficiency, may facilitate the effort to prevent insufficiency by appropriate means. The method of experimental investigation described herein is a step in that direction.

The manifestations of adrenal insufficiency described earlier (Rogoff and Stewart [11]) occurred in these experimental animals, but they were milder and appeared more insidiously than

9. Rogoff, J. M., and Stewart, G. N.: (*a*) Demonstration presented at the annual meeting of the American Physiological Society, Cleveland, 1925; (*b*) Science **66**: 327, 1927; (*c*) Am. J. Physiol. **84**:660, 1928; (*d*) **86**: 25, 1928.

10. Rogoff, J. M.: J. A. M. A. **99**:1309, 1932; J. Clin. Endocrinol. **2**:36 and 43, 1942.

11. Rogoff, J. M., and Stewart, G. N.: (*a*) Am. J. Physiol. **78**:683, 1926; (*b*) footnote 9a.

those in adrenalectomized animals except when acute crises developed. As demonstrated in some of the protocols, the earliest symptoms to develop are referable to the alimentary tract. If the symptoms are associated with chronic or subacute insufficiency, they respond readily to administration of adrenal cortex extract; when they rapidly merge with the manifestations of an acute crisis, treatment with cortical extract is less effective unless combined with intravenous administration of isotonic solution of sodium chloride.

The relative merits of prevailing theories of adrenal function need not be discussed here. Extensive experimental and clinical investigations on this subject have not altered my original view that adrenal cortical insufficiency represents a severe complex metabolic disturbance and cannot be explained satisfactorily as a realtively simple alteration involving carbohydrate metabolism, chloride deficiency, sodium-potassium balance or water balance. Each and all of these and other disturbances can be explained better as a part of the much more complex physiologic derangement which occurs in consequence of failure or inadequacy of adrenal cortical function. Nor is the recently proposed view that the adrenal cortex does not elaborate a specific hormone but rather numerous steroids, each of which has a special function, the sum of all of them representing the function of the adrenal cortex (Kendall [12]), tenable as a physiologic concept.

The possibility of chemical disruption of interrenalin into component steroids or other simpler compounds does not indicate that each of such components represents a separate hormone capable of exercising a distinct special endocrine function. For example, it would not be assumed that each of the component polypeptides or amino acids derived from chemical disruption of the protein in the molecule of iodothyroglobulin represents a different hormone and performs a distinctly special endocrine function.

Not only can severe experimental adrenal insufficiency or clinical Addison's disease occur without significant diminution in the level of sodium chloride in the blood but, as demonstrated in the preceding protocols, blood sugar does not fall below the lower level of the normal range when it declines at all. While administration of salt is beneficial in adrenal insufficiency, that apparently is chiefly because of the consequent increase in the intake of water, which facilitates the elimination of toxic metabolites, rather than the direct effect of the salt. For it has been possible to resuscitate adrenalectomized dogs from coma and to ameliorate other symptoms of insufficiency by intravenous administration of isotonic dextrose solution without salt.

It has been indicated that the most common and apparently most significant change in the blood is an increase in nonprotein nitrogen reflecting an increase in the "undetermined fraction." The increase in total serum calcium (Rogoff and Stewart [9c, d]) is of special interest in that a large proportion of the animals with chronic or subacute adrenal insufficiency, especially those surviving longer periods, presented enlarged parathyroid glands at autopsy. Further evidence indicating an interrelationship between activity of the parathyroid glands and function of the adrenal cortex can be found in the experiments of Schour and Rogoff [13] and of Collip.[14]

Marked congestion of the pancreas was found at autopsy in acute adrenal insufficiency following adrenalectomy (Rogoff and Stewart [11a]). It was suggested that this observation may have a bearing on the early development of anorexia and especially on that of the commonly occurring aversion to fatty food. This interesting but unexplained condition of the pancreas was observed repeatedly in animals with subacute and chronic as well as acute adrenal insufficiency on inspection of the pancreas at exploratory laparotomy or during operations for religation or excision of adrenal glands. Sometimes a congested pancreas was seen at one exploratory operation, when symptoms were present, and later the organ appeared normal when inspected during a succeeding laparotomy performed when the animal had recovered from the manifestations of adrenal insufficiency.

In general it can be stated that the pathologic changes in the adrenal cortex paralleled in degree the severity of the symptoms; also, that the changes in the blood and the condition of other organs, e. g., the pancreas, were definitely related to the adrenal pathologic changes, appearing when degeneration was active and disappearing or becoming less marked as regeneration of the cortex exceeded degeneration. Complete recovery or extensive regeneration of the gland was associated with complete recovery from symptoms, from the acute or subacute changes in the pancreas and from the chemical changes in the blood.

SUMMARY

The production of experimental Addison's disease has been accomplished in cats and dogs by subtotal vascular occlusion of the adrenal glands, which induced chronic and subacute as

12. Kendall, E. C.: J. A. M. A. **116**:2394, 1941.

13. Schour, I., and Rogoff, J. M.: Am. J. Physiol. **115**:334, 1935.

14. Collip, J. B.: Am. J. Physiol. **76**:472, 1926.

well as acute adrenal insufficiency. The same process may occur in human beings, inducing Addisons' disease, as an accidental consequence of surgical denervation or partial ablation of the adrenal glands practiced as a therapeutic procedure. Manipulation of the adrenal glands can easily cause vascular thrombosis and occlusion.

In the experimental animals the procedure usually led to anemic infarction, degeneration and necrosis of the gland, with regeneration, fibrosis and atrophy, comparable or identical with the pathologic changes designated as "cytotoxic atrophy" of the adrenal glands often encountered in human Addison's disease. Selective degeneration of the cortex, leaving the medulla intact, was readily produced in a large number of the experimental animals.

The clinical manifestations, the chemical changes in the blood and other manifestations of adrenal insufficiency produced in the animals were the same in general as occur in Addison's disease and, more severely, in adrenalectomized animals. The most characteristic of the blood changes is an increase in the "undetermined fraction" of the nonprotein nitrogen. Congestion of the pancreas commonly occurs in the subacute and the acute stage of adrenal cortical insufficiency. Experimental chronic and subacute adrenal insufficiency is usually associated with enlargement of the parathyroid glands; this is of interest in relation to the increase in total serum calcium that occurs.

When degeneration exceeded regeneration, acute crises, often death, resulted. Recovery ensued if regeneration was more active than degeneration, the necrotic portion of the gland being replaced by fibrous tissue; sometimes calcification occurred.

These experiments constitute a method for fundamental investigations of the adrenal cortex from the standpoints of physiology and pathology.

My assistant, Miss E. Nola Nixon, gave aid in the surgical operations and prepared the histologic material. Dr. D. P. Seecof assisted in the interpretation of the pathologic changes in the adrenal glands and in the preparation of the photomicrographs.

Case Reports

LYMPHANGIOMA OF THE JEJUNUM

I. Darin Puppel, M.D., and Lloyd E. Morris Jr., M.D., Columbus, Ohio

Lymphangioma is one of the least frequently encountered types of benign tumor of the small intestine. A comparative study of 35 cases of benign tumor of the small intestine in the files of the Mayo Clinic, reported by Rankin and Newell [1] in 1933, revealed no case of lymphangioma. Adenoma, myoma, fibroma and lipoma were the most frequent types. Hemangioma occurred twice in their series. In 1932 Raiford [2] cited 37 cases of benign tumor of the small intestine in 11,500 postmortem examinations and was able to add 13 from the material in the department of surgical pathology at the Johns Hopkins Hospital, which included 45,000 surgical specimens. Among these, specimens of adenoma, fibroma, myoma, aberrant pancreatic rest, argentaffin tumor and lipoma were most frequent. There were 3 specimens of hemangioma and 1 of lymphangioma. The latter was obtained at autopsy from a Negro man of 52 years. The tumor was asymptomatic and was located in the jejunum. The patient died of carcinoma of the rectum. The tumor diagnosed as lymphangioma consisted of three opaque grayish nodules measuring 2 to 3 mm. in diameter and containing whitish fluid. Microscopic examination revealed a cystic structure with sacs lined by endothelium and separated by delicate septums.

The following report concerns a case of lymphangioma of the jejunum which was treated successfully by resection.

REPORT OF A CASE

A white woman of 55 years entered University Hospital, in Columbus, Ohio, on Oct. 7, 1942. She complained principally of anemia, extreme weakness and melena. About twenty years prior to admission she underwent a pelvic operation for removal of an ovarian growth of unknown nature. After that there were several episodes of anemia accompanied by pallor and extreme weakness. Elsewhere, throughout those years she was given liver extract by injection, iron by mouth and a diet high in animal protein. Later, blood transfusions afforded temporary benefit. During several months before admission she complained of pain along the right costal margin.

Examination.—The temperature was 100.2 F., the pulse rate 100, the respirations 20 and the blood pressure 112 systolic and 70 diastolic. The patient was a well developed but poorly nourished white woman who appeared pale and chronically ill. The turgor of the skin was poor. The liver was palpable 3 fingerbreadths below the right costal margin. The spleen was barely palpable below the left costal margin. A large tender mass was palpable in the right upper quadrant of the abdomen. Otherwise the physical examination gave negative results.

The red blood cell count on Sept. 10, 1942 was 1,160,000. The hemoglobin content of the blood was 2.7 Gm. per hundred cubic centimeters. The reticulocyte percentage was 13.4. The red cells showed slight anisocytosis, poikilocytosis and hypochromia. The white cell count was 2,750, with neutrophils 64 per cent, myelocytes of group C 2 per cent, lymphocytes 16 per cent, monocytes 14 per cent and basophils 4 per cent. The platelet count was 278,000. A few small macrocytes were noted. A study of the bone marrow showed no disturbance. The blood urea nitrogen was 12 mg. per hundred cubic centimeters. The gastric content on analysis was essentially normal. Roentgenographic studies of the stomach and the duodenum during ingestion of a barium sulfate meal and of the colon during a barium sulfate enema disclosed nothing abnormal. Repeated examinations of the stools for occult blood while the patient was maintained on a meat-free diet gave strongly positive results. Retrograde pyelography and ureteral catheterization revealed no abnormality.

Hospital Course and Operation.—Renal disease and primary blood dyscrasias were ruled out. After careful study, carcinoma of the intestine was suspected. Repeated blood transfusions failed to increase the red blood cells and the hemoglobin appreciably. Exploratory laparotomy was therefore done on October 22. Examination of the colon on the right side was undertaken first and showed nothing suggestive of cancer. The tender mass in the right upper quadrant of the abdomen proved to be a normal kidney, ptosed as were all the viscera. The entire colon was then palpated and as much of it as possible inspected, but no evidence of disease was found. The stomach, the gallbladder, the pancreas, the duodenum and the liver were explored but were normal. It was noted that there was slight dilatation of a loop of jejunum. Palpation revealed a small soft mass in this loop. Three or four yellowish nodules showed through the serosa of this area. The mass, as well as about 4 inches (10 cm.) of intestine on either end of the mass, was resected. The ends were closed and a lateral anastomosis was effected. The abdomen was then closed. A blood transfusion of 500 cc. was given during the operation and a similar transfusion immediately thereafter. Another one was administered on the first postoperative day. On November 9 the red blood count was 4,200,000 and the hemoglobin content of the blood 12.3 Gm. per hundred cubic centimeters. The white cell count was 3,900, with neutrophils 60 per cent, lymphocytes 34 per cent, monocytes 1 per cent, eosinophils 4 per cent and basophils 1 per cent. The examination of the stools for occult blood on November 8 showed none.

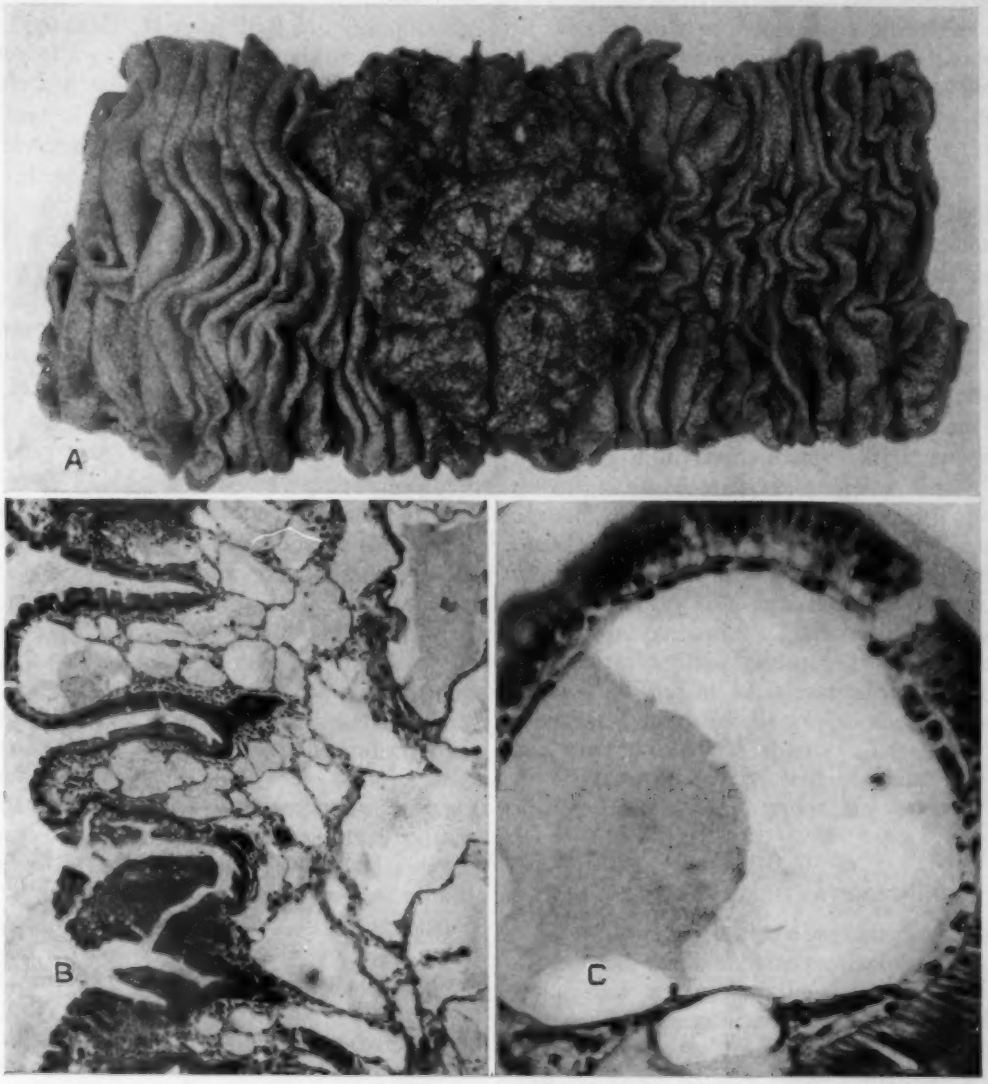
From the Departments of Research Surgery and Pathology, Ohio State University.

1. Rankin, F. W., and Newell, C. E.: Surg., Gynec. & Obst. **57**:501, 1933.

2. Raiford, T. S.: Arch. Surg. **25**:122 and 321, 1932.

The patient made an uneventful recovery and was discharged on November 12. She was seen at intervals during seventeen months following resection. Clinically she appeared to be relieved of her disease. The red blood cell count on March 9, 1944 was 4,100,000. The hemoglobin content was 82 per cent of normal. The white cell count was 6,800, with 69 per cent neutrophils, 24 per cent lymphocytes, 4 per cent monocytes and 3 per cent eosinophils.

Pathologic Examination of Specimen.—An excised portion of jejunum measuring 25 cm. in length was examined. On the mucosal surface was a large fungating mass which occupied practically the entire circumference of the intestine (*A* in figure). It measured 6 cm. in diameter. It was elevated 1.5 cm. above the mucosa. It was polypoid, soft and velvety in consistency and apparently was made up of numerous confluent small nodules. The surface presented a dirty gray appearance. Around the margins were a few small yellowish areas in which the folds of mucous membrane were apparently infiltrated with lipoid material. The serosal surface at the base of the tumor presented a subserous fluid, which apparently was edematous in character, and a dark mottled bluish background. In the margin of the serous surface of the tumor were isolated small yellow nodules. Microscopic examination revealed a cavernous lymphangioma (*B* in figure). Cystic dilatations of varying size separated by thin septums and lined by endothelium were prominent. They contained a homogeneous pink-staining material through which were scattered occasional epithelial or blood cells.



A, lymphangioma of the jejunum. *B*, cavernous lymphangioma. Note the cystic dilatations of varying size lined by endothelium and separated by thin septums. They contain coagulated lymph, through which are scattered occasional epithelial or blood cells. *C*, high power photomicrograph of cavernous lymphangioma.

COMMENT

Preoperative clinical recognition of a noncancerous tumor of the small intestine is at best a difficult task. An exact preoperative diagnosis of lymphangioma of this region is unknown. The symptoms of a tumor of the small

intestine are mainly those of obstruction.[3] There is a group of tumors such as that in our case which do not obstruct. A tumor belonging to this group is more obscure and presents a more difficult diagnostic problem. Here roentgenography rarely offers positive evidence of a tumor and seldom helps in the recognition of one. Gross hemorrhage may rarely appear in the stools. Smaller hemorrhages, such as occurred in the present case, are more frequent, and occult blood may be detected by chemical tests.

The subsequent history of this patient over seventeen months following resection suggests that there are no other bleeding lesions along the gastrointestinal tract. No melena has occurred since operation. The anemia has completely subsided.

3. Puppel, I. D., and Morris, L. E., Jr.: Am. J. Surg. **66**:113, 1944.

General Reviews

THE PARASITIC LACTOBACILLI

A CRITICAL REVIEW OF THEIR CHARACTERISTICS AND PARASITIC EFFECTS AND OF THE BACTERIOLOGY OF DENTAL CARIES

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The parasitic lactobacilli are conspicuous members of a group of micro-organisms that can be cultivated from areas of mucous membrane by the use of a selective medium. The medium contains certain growth-stimulating substances and a utilizable sugar such as dextrose in addition to the usual nutrient constitutents and is titrated to a relatively low p_H—generally 5.0. This level of hydrogen ion activity inhibits the growth of most bacterial species in a source such as saliva or feces but permits the development of a restricted range of micro-organisms. In addition to lactobacilli, yeasts are frequently observed in such cultures, and less constantly staphylococci, streptococci and such typically air-borne saprophytes as micrococci and sarcinae. These are the aciduric (acid-enduring) micro-organisms, so named by Kendall.[1] The lactobacilli are the most prominent numerically and probably the most important members of the group. Not only do they outnumber the others, but they seem to have the greatest capacity in the group both to produce and to withstand high hydrogen ion concentrations in their environment. Their acidogenic and aciduric capacity is indeed their outstanding property. Aside from their association with dental caries the lactobacilli are not known to be pathogenic. On the contrary, they have been assigned beneficial roles on mucous membranes in regions other than the mouth, notably in the vagina. It may be emphasized that their effects as parasites on mucous membranes, whether harmful or beneficial, seem directly traceable to their ability to elaborate unusually high concentrations of acid and to continue to live in such an acidified environment.

CLASSIFICATION

The lactobacilli are a varied group with both parasitic and saprophytic members. The saprophytic forms are included with the lactic streptococci among the lactic acid bacteria, which are of considerable economic and industrial importance but are concerned here only incidentally. The parasitic forms are themselves heterogeneous. The classification of the group has not yet been generally agreed on, and several aspects of it are matters of controversy.

The genus Lactobacillus is grouped by Bergey and co-workers[2] in the family Lactobacteriaceae, which includes the streptococci and the pneumococci. The lactobacilli are gram-positive rods of variable morphologic aspect. They are nonmotile, do not form spores, fail to liquefy gelatin and neither reduce nitrates nor form indol. They produce lactic acid from carbohydrates. Only certain saprophytic species produce gas. Most species grow under either aerobic or anaerobic conditions, with the latter often preferred. Some are more or less strict anaerobes. Some saprophytic species are thermophilic. Bergey lists fifteen species, only two of which are parasitic: Lactobacillus acidophilus and Lactobacillus bifidus. It is curious that both are listed as intestinal forms and that no mention is made by Bergey of the lactobacilli of the mouth and the vagina. In the following descriptions the oral and vaginal forms are included tentatively under the species named L. acidophilus.

LACTOBACILLUS ACIDOPHILUS

L. acidophilus is the designation used here to include all aerobic parasitic lactobacilli. These organisms are nonmotile rods without spores, subject to considerable variation in size and shape. They are gram positive, but even young cultures often include many individual rods that are easily decolorized, and older cultures may appear entirely gram negative. Their surface colonies generally do not exceed a diameter of 1 to 1:5 mm. and in appearance range widely from raised smooth opaque white ones like small colonies of Staphylococcus albus through smooth translucent streptococcus-like colonies to inter-

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1. Kendall, A. I.: J. M. Research **22**:153, 1910.

2. Bergey, D. H., and others: Bergey's Manual of Determinative Bacteriology, ed. 5, Baltimore, Williams & Wilkins Company, 1939, p. 323.

mediate and rough ones. The smooth opaque colonies are seen especially on enriched mediums (e. g., tomato agar) and the translucent ones on ordinary dextrose agar, while the rough ones may appear on any solid medium.

L. acidophilus does not grow in carbohydrate-free mediums. Limited growth can be obtained with ordinary infusion peptone mediums containing dextrose, but best results require the use of improved peptones (e. g., "neopeptone") and the further addition of supplements rich in vitamin B fractions, such as liver extract, yeast extract or tomato juice. Even on such enriched mediums growth is often poor, and many freshly isolated strains can be maintained only by frequent subculture with use of large inoculums. The mediums most commonly used for the isolation of lactobacilli from the mouth are 1 per cent dextrose meat infusion broth of p_H 5.0 [3] and Hadley's [4] modification of the tomato agar of Kulp and White.[5]

Optimal growth of L. acidophilus occurs at 37 C. Weiss and Rettger [6] found that growth was generally optimal in the p_H range 5.8 to 6.6 Longsworth and MacInnes [7] obtained maximal growth at p_H 6.0 under conditions of continuous p_H control. Growth occurs, however, over a much wider range of p_H, between about 4.0 or lower and 8.0 or more. Acid is produced without gas from a wide range of carbohydrates. In standard dextrose broth final p_H values as low as 3.5 have been obtained, but many strains, particularly those derived from rough colonies, give much higher final values.

Many attempts have been made to subdivide these organisms into homogeneous categories, but thus far none has met with unqualified success. The primary bases of these attempts, as detailed subsequently, include: (*a*) colony form in deep agar; (*b*) colony form on the surface; (*c*) type of lactic acid produced; (*d*) growth capacity and final p_H, and (*e*) serologic means, correlated with morphologic features of the colony and with fermentation reactions.

Colony Form in Deep Agar.—Rettger and Horton [8] distinguished two types of L. acidophilus, one of which, the X type, produced fuzzy colonies with fine filamentous outgrowths, while the other, the Y type, grew in compact sand-grain-like colonies, both in poured plates. These types would correspond with rough and smooth forms, respectively, in the more modern terminology. Kulp and Rettger [9] found that both types appeared among intestinal lactobacilli. They regarded the X type as more characteristic but noted that all gradations between the two were present in their cultures and that organisms isolated from extreme X type colonies frequently yielded Y colonies in subsequent platings. The principal virtue of the distinction has been its relation to intestinal "acidophilus therapy," which is discussed later. Rettger, Levy, Weinstein and Weiss [10] have urged using the X form for this purpose, but the reason for doing so seems to have been one of assurance in identification rather than any demonstrated superiority of the X over the Y type. In the light of modern knowledge of bacterial variation there is at all events little reason to believe that a distinction of this kind can be valid in itself for the separation of types within a species.

Colony Form on Surface.—Differences in colony form as a general rule, whether used to distinguish different species or variants within a single species, are most clearly seen in colonies growing on the free surface of a solid medium. Attempts to subdivide the aerobic lactobacilli on this basis have been made particularly with strains derived from the mouth. Hadley, Bunting and Delves [11] and Hadley and Bunting [12] used the colony form on the surface of casein digest, sodium oleate agar as a primary criterion for subdividing mouth lactobacilli into four groups. Hadley [4] later described these groups as they appear on tomato agar of p_H 5.0, which has come to be widely used for the isolation of oral lactobacilli. Hadley's groups are defined as follows:

Group I appears as small (about 0.8 mm.) raised smooth opaque colonies containing rather uniform small coccobacilli or curved gram-positive rods which tend to lie in pairs with the concave sides approximating. Gram-negative bacilli are not conspicuous in young cultures. Chains may appear in broth. The colonies are said to be very stable, although rough variants containing long chains and filaments could be

3. Jay, P., and Voorhees, R. S., Jr.: Dent. Cosmos **69**:977, 1927.

4. Hadley, F. P.: J. Dent. Research **13**:415, 1933.

5. Kulp, W. L., and White, V.: Science **76**:17, 1932.

6. Weiss, J. E., and Rettger, L. F.: J. Bact. **28**:501, 1934.

7. Longsworth, L. G., and MacInnes, D. A.: J. Bact. **29**:595, 1935.

8. Rettger, L. F., and Horton, G. D.: Zentralbl. f. Bakt. (Abt. 1) **73**:362, 1914.

9. Kulp, W. L., and Rettger, L. F.: J. Bact. **9**:357, 1924.

10. Rettger, L. F.; Levy, M. N.; Weinstein, L., and Weiss, J. E.: Lactobacillus Acidophilus and Its Therapeutic Application, New Haven, Conn., Yale University Press, 1935.

11. Hadley, F. P.; Bunting, R. W., and Delves, E. A.: J. Am. Dent. A. **17**:2041, 1930.

12. Hadley, F. P., and Bunting, R. W.: J. Am. Dent. A. **19**:28, 1932.

derived from such smooth colonies. The strains of group I produced an average hydrogen ion concentration of p_H 3.8 in dextrose broth, curdled milk in one to two days and gave variable fermentation reactions.

Group II is comprised of somewhat larger colonies (1.0 to 1.2 mm.) which are smooth, white and opaque and usually are more raised than the colonies of group I. Both in agar and in broth the organisms are straight rods that tend to lie in palisade arrangement and include both gram-positive and gram-negative rods even in young (forty-eight hour) cultures. The colonies are described as somewhat unstable, tending easily to form rough variants containing filaments of different lengths. They are said to be less actively acidogenic than those of group I, giving an average final p_H of 4.1, coagulating milk slowly and fermenting a narrower range of carbohydrates.

Group III includes forms intermediate between the foregoing smooth types and the true rough variants. The colonies are as large as 1.5 mm., flat, translucent and irregular in both surface and margin. Deep colonies have been said to correspond with Rettger's X type. Gram stains show considerable morphologic irregularity, with rods and filaments, many of the latter curved and swollen, and with both gram-positive and gram-negative forms. The acidogenic powers were similar to or weaker than those of group II.

Group IV is represented by a rough colony, larger than the others, with a very filamentous edge. Its contained organisms are thick bacilli and long winding filaments with "curled" and "wavy" forms. Hadley and Bunting [12] stated that groups I, II, and III can be transformed in vitro into group IV.

In practice, forms intermediate between any two of the four groups are commonly observed. Colonies of the groups described are nevertheless common, and the Hadley classification has found a degree of acceptance and has been used as a basis for a more satisfactory subdivision.

Type of Lactic Acid Produced.—The classification proposed by Curran, Rogers and Whittier,[13] of which an important basis was the observation that one group produces inactive lactic acid and another dextrorotatory lactic acid, seems to have been generally overlooked in later literature and deserves mention. These workers studied 103 strains of lactobacilli derived from the mouth, the intestine and the vagina of the human being, from the intestine of the rat and from miscellaneous sources, including commercial preparations of L. acidophilus and other nonparasitic habitats. A few of the strains appear to have been Lactobacillus bulgaricus and other saprophytes. The strains were divided into groups A, B and C.

The strains of group A produced inactive lactic acid only. The colonies were chiefly rough ones, with some admixture of smooth ones. Other differential features were inability to grow at 20 C., production of volatile acids to the extent of 12 to 20 per cent, fermentation of raffinose but not of mannite, and ability to grow in suitable mediums containing phenol in concentrations of 1:250 to 1:400. All criteria except the first (inactive lactic acid) were subject to some degree of variation.

The strains of group B produced a mixture of inactive and dextrorotatory lactic acid. The colonies were chiefly smooth ones, with some admixture of rough ones; in exceptional cultures they were all rough. All cultures grew at 15 C. and some at 10 C. They produced volatile acids up to 4 to 12 per cent, usually fermented mannite but not raffinose and were inhibited by phenol in the concentration of 1:400.

The strains of group C produced inactive lactic acid only but grew at 20 C. The colony form was intermediate, as were the morphologic and biochemical characteristics.

The statistical nature of this classification is apparent and seems significant. The descriptions represent average types within a range of continuous variation rather than well marked categories. Curran and his collaborators suggested that group A represented the typical L. acidophilus; 17 of 23 intestinal strains fell into this group, a higher proportion than in any other source group. Yet the strains from all sources were distributed among all the groups without marked preponderance. Fourteen of 24 oral strains, for example, fell in group B, 8 in group A and 2 in group C. It may be noted that Kopeloff and Kopeloff [14] observed that rough colonies of L. acidophilus produced inactive lactic acid, whereas smooth ones produced the dextrorotatory acid. These data suggest that neither the colony form nor the optical activity of the acid produced can be accepted as an adequate criterion for classification.

Growth Capacity and Final p_H.—It is apparent that the foregoing attempts to subdivide L. acidophilus have yielded groups which have features in common, suggesting that they might be integrated, and which are of doubtful validity in view of their dependence on variations to

13. Curran, H. R.; Rogers, L. A., and Whittier, E. O.: J. Bact. **25**:595, 1933.

14. Kopeloff, L. M., and Kopeloff, N.: J. Bact. **33**: 331, 1937.

which any given strain appears to be subject. Another classification on a related basis, and with similar defects, was suggested by Rosebury [15] and amplified by Sullivan, Still and Goldsworthy.[16] In the former instance 17 strains of lactobacilli derived from human and rat mouths and 2 from intestines were classified into three groups on the basis of ability to lower the p_H of a weakly buffered dextrose broth titrated to different initial p_H levels between 4 and 6.5. Group I, including most of the strains with smooth and intermediate colonies from the human mouth and intestine, produced final p_H values below 3.5. Group II produced final p_H values between 3.5 and 4.0, and group III gave values above 4.0. Groups II and III were composed principally of strains with intermediate and rough colonies and included all the strains from rat mouths. It was noted that ability to lower the p_H was correlated with the growth capacity in stock dextrose broth, the most actively growing strains being generally the most active acid producers. Sullivan and his collaborators studied 103 oral strains of lactobacilli and classified them into two groups on the basis of final p_H and titratable acid in dextrose broth. Their S. A. (strongly acidogenic) group included 67 strains; their W. A. (weakly acidogenic) group, 36. They did not attempt to correlate colony form and morphologic observations with these findings and did not present data on the relative growth capacity of their strains. They argued that their S. A. strains are probably more important than the others in the causation of dental caries (see also Sullivan [17]). This point is relevant here in that it reflects an apparently gratuitous assumption, made by these workers and others, that the characteristics of a bacterial strain observed in vitro provide a faithful index of its characteristics in vivo. It does not follow that an organism which grows abundantly and is biochemically active in artificial culture necessarily behaves in the same way in its natural habitat. The reverse may conceivably be true: Organisms which are well adapted to a parasitic habit of life may grow poorly and appear inactive as saprophytes, and vice versa. Much of the emphasis on smooth active lactobacilli isolated from the mouth seems to have depended on the fact that such strains were easier to manage in culture than the poorly growing rough strains which almost invariably accompany and frequently outnumber the former.

15. Rosebury, T.: J. Bact. **24**:321, 1932.
16. Sullivan, H. R.; Still, J. L., and Goldsworthy, N. E.: J. Dent. Research **18**:513, 1939.
17. Sullivan, H. R.: J. Dent. Research **18**:525, 1939.

Serologic and Correlated Characteristics.—Serologic studies of L. acidophilus reported before 1939 (Harrison, Zidek and Hemmens [18]) were devoted mainly to comparisons of oral and intestinal strains; they found the group to be generally heterogeneous and did not advance the problem of its finer classification. The same has been true of fermentation reactions. By combining these two means, however, and correlating them with morphologic criteria, Harrison and his co-workers have brought this question nearer to its eventual solution. These workers have studied only oral strains, but since source does not appear to be a valid basis for subdivision of the parasitic lactobacilli, their findings may have wider application.

Harrison [19] first classified oral strains of lactobacilli into two groups on the apparently rigid basis of coagulation and reduction of litmus milk. Group A comprised 75 strains which produced these changes; group B consisted of 26 strains which failed to produce them. Group A, which presumably corresponds with the strongly acidogenic groups of the previous workers, consisted principally of Hadley group I strains (42 of 75) but also included 22 of group II and 11 of group III. All these strains fermented mannite. The strains of group B were more evenly distributed among the Hadley groups, 10 falling in group I, 5 in group II and 11 in group III. Only 6 of the 26 strains of group B fermented mannite. Fermentation of sorbitol, raffinose and dextrin varied in both groups and was used to distinguish subgroups.

The framework for what may turn out to be a solid foundation has been placed under this classification by serologic studies in which methods were used similar to those applied most successfully to hemolytic streptococci by Lancefield.[20] Harrison, Zidek and Hemmens [18] found that they could obtain water-soluble carbohydrate fractions from lactobacilli by repeated heating in sixteenth-normal hydrochloric acid. The supernatant fluid after neutralization was repeatedly precipitated with alcohol and the precipitate redissolved in distilled water until it was completely water soluble. Using antiserums prepared against 10 strains of group A and against 7 strains of group B as previously defined, Harrison [21] found that carbohydrate fractions from 168 of 224 strains of group A (75 per cent) reacted positively with group A antiserums, whereas none of 85 such extracts of

18. Harrison, R. W.; Zidek, Z. C., and Hemmens, E. S.: J. Infect. Dis. **65**:255, 1939.
19. Harrison, R. W.: J. Infect. Dis. **65**:142, 1939.
20. Lancefield, R. C.: J. Exper. Med. **57**:571, 1933.
21. Harrison, R. W.: J. Infect. Dis. **70**:69, 1942.

strains of group A reacted with group B serums. Group B was much more heterogeneous serologically. These findings suggest that a classification of L. acidophilus may be feasible along lines currently being used for the subdivision of viridans streptococci of the mouth, which also seem to fall into a homogeneous group (Streptococcus salivarius) that includes a high percentage of isolated strains and into a heterogeneous group distinct from the former (Streptococcus mitis) but variable within itself (Sherman, Niven and Smiley [22]). Present indications are that such a homogeneous group of L. acidophilus may include a considerable number of smooth, actively acidogenic strains. At present, however, such morphologic or biochemical criteria cannot be used alone as a basis for a fully satisfactory classification.

LACTOBACILLUS BIFIDUS

The species designation L. bifidus is applied to those parasitic lactobacilli that require anaerobiosis for primary isolation. Some forms are strict and permanent anaerobes, while others become adapted to aerobic growth after repeated subculture. The species thus seems to intergrade with L. acidophilus. L. bifidus resembles the aerobic forms in staining properties but is distinguished by an even greater morphologic variability of the cells. Bifurcated and clubbed forms develop in some cultures and appear with particular frequency in infant feces. The organism seems to be essentially an intestinal parasite. Its occurrence in the mouth has been described (Howe and Hatch [23]), but oral strains have not been extensively studied. Boventer [24] reported that L. acidophilus and L. bifidus are serologically distinct. Rettger, Levy, Weinstein and Weiss,[10] on the other hand, could not distinguish the two groups on biochemical or serologic grounds and considered them to be closely related if not identical. They were unable to find bifid forms in pure cultures except in Veillon tubes (Veillon and Zuber [25]) and noted that L. acidophilus produced similar forms under these conditions. At that time they seem to have worked only with facultative strains, but later Weiss and Rettger [26] confirmed the observation of Eggerth [27] and others that strictly anaerobic forms exist in the intestinal tract. It was thus suggested that at least two varieties of L. bifidus can be distinguished, one of which (type I), the classic form originally described by Tissier,[28] is anaerobic only on primary isolation, while the other (type II), corresponding with forms previously grouped with the genus Bacteroides, is permanently anaerobic. The two types were found to differ serologically and in other characteristics. Lewis and Rettger [29] reported that the properties of 26 intestinal strains of anaerobic non-spore-bearing nonmotile gram-positive rods all linked them with the lactobacilli. Only four of these strains showed branching forms. The strains of Lewis and Rettger were shown by King and Rettger [30] to be related both to L. bifidus type II and to the "Bacteroides bifidus" of other workers by means of complement fixation tests and intradermal tests in sensitized rabbits and also on the basis of cyanide inhibition tests (see the following section).

The cutaneous reactions in rabbits, which were of the delayed type, gave particularly sharp distinctions between the anaerobic lactobacilli, on one hand, and, on the other, the anaerobic gram-negative rods (true Bacteroides) and the aerobic intestinal lactobacilli, but data on cutaneous reactions for type I L. bifidus were not presented. The distinction between L. bifidus type I and L. acidophilus thus seems to rest only on a difference in tolerance of oxygen. In dextrose agar shake cultures L. acidophilus grows throughout the tube, whereas L. bifidus does not grow in the upper centimeter or so.

METABOLISM

The lactobacilli parallel the streptococci closely in their metabolism, both in their relation to oxygen and in their fermentation (Rosebury [31]). They do not require free oxygen for growth, and L. acidophilus seems to be largely indifferent to the presence of this element in the medium (Longsworth and MacInnes [32]), while the strictly anaerobic varieties of L. bifidus are inhibited by it. L. acidophilus produces traces of peroxide (McLeod, Gordon and Pyrah [33]), and it may be noted that some strains may induce

22. Sherman, J. M.; Niven, C. F., and Smiley, K. L.: J. Bact. **45**:249, 1943.

23. Howe, P. R., and Hatch, R. E.: Dent. Cosmos **59**:961, 1917.

24. Boventer, K.: Zentralbl. f. Bakt. (Abt. 1) **142**: 419, 1938.

25. Veillon and Zuber: Arch. de méd. expér. et d'anat. path. **10**:517, 1898.

26. Weiss, J. E., and Rettger, L. F.: J. Infect. Dis. **62**:115, 1938.

27. Eggerth, A. H.: J. Bact. **30**:277, 1935.

28. Tissier, H.: Recherches sur la flore intestinale des nourrissons, Thesis, Paris, George Carré & C. Naud, 1900, p. 253.

29. Lewis, K. H., and Rettger, L. F.: J. Bact. **40**: 287, 1940.

30. King, J. W., and Rettger, L. F.: J. Bact. **44**:301, 1942.

31. Rosebury, T.: Medicine **23**:249, 1944.

32. Longsworth, L. G., and MacInnes, D. A.: J. Bact. **31**:287, 1936; **32**:567, 1936.

33. McLeod, J. W.; Gordon, J., and Pyrah, L. N.: J. Path. & Bact. **26**:127, 1923.

greening of blood agar.[34] Lactobacilli require riboflavin for growth and contain flavoproteins.[35] They do not contain catalase or cytochromes.[36] These characteristics—lack of iron-porphyrin enzyme systems, presence of flavoproteins, and either no utilization of oxygen or utilization of it in small amounts with production of H_2O_2—are common to the lactic acid bacteria. King and Rettger[30] found that concentrations of potassium cyanide of 1:2,000 and 1:1,000 inhibited the growth of L. bifidus (both type I and type II) in the depths of dextrose agar shake cultures but not the growth nearer the surface. L. acidophilus and L. bulgaricus were not affected by these concentrations of cyanide under similar conditions. Sevag and Shelburne[37] have reported parallel evidence in the streptococci of a cyanide-sensitive enzyme system of unknown nature.

The energy-yielding mechanisms of the lactobacilli, like those of the streptococci, seem to be essentially anaerobic, with lactic acid being produced from carbohydrates alone ("homofermentative") or accompanied by small amounts of volatile acids, alcohol and carbon dioxide ("heterofermentative"). Lactic acid has been found most commonly as the major product of the fermentation of sugars by L. acidophilus and other lactobacilli, although Dodds[38] has reported the production of malic acid, and Nakai[39] found succinic acid in cultures of oral L. acidophilus. According to Kopeloff and Kopeloff,[14] the lactic acid produced by rough strains of L. acidophilus is inactive, while that produced by smooth strains is dextrorotatory. The mechanism of the production of lactic acid from dextrose seems to parallel that for muscle, involving phosphorylation, the formation of triose phosphates and dephosphorylation of pyruvic acid, which reenters the cycle to oxidize one of the triose phosphates and is itself reduced to lactic acid (Werkman[40]).

The nutrition of the lactobacilli has been studied chiefly in relation to the saprophytic lactic acid bacteria, including various species of Lactobacillus and Streptococcus lactis. Snell, Strong and Peterson[41] and Snell and Peterson[42] have been able to grow these forms in nonsynthetic mediums containing salts, dextrose and various amino acids, including tryptophane and cystine, with the addition of pantothenic acid, riboflavin, pyridoxine and nicotinic acid. Additional unidentified growth substances are also required, and the lactobacilli have not yet been grown in chemically defined mediums. Hill and Kneisner[43] have reported that oral lactobacilli require dextrose and pantothenic acid. A concentration of dextrose of 0.15 per cent was sufficient for maximum growth. The pantothenic acid requirement for optimal growth varied from 0.018 to 0.07 micrograms per cubic centimeter, depending on the strain, with the variation not well correlated with type by the Hadley classification.

INTERRELATIONSHIPS AMONG THE LACTOBACILLI

The relationship of L. acidophilus to L. bifidus has been considered with the description of the latter organism, where it appears that type II L. bifidus is distinct but that type I intergrades with the aerobic forms. One is concerned here with three questions: (*a*) the relationship of L. acidophilus to saprophytic lactobacilli, particularly L. bulgaricus; (*b*) the interrelationships of forms described as L. acidophilus, obtained respectively from the vagina, the intestine and the mouth, and (*c*) the interrelationship of lactobacilli and streptococci. An additional question, the relationship of the anaerobic lactobacilli to the anaerobic actinomycetes, is dealt with elsewhere.[44]

L. Acidophilus and L. Bulgaricus.—These two species of lactobacilli intergrade and may be difficult to distinguish. One is characteristically a parasite of mucous membranes and the other is a saprophyte in milk and milk products, but it seems not impossible that either may be capable of giving rise to the other by adaptation to the appropriate environment. Following sections show that L. bulgaricus does not survive implantation in the human intestine. This finding, in view of its apparent importance in intestinal therapy, was made the chief basis for distinguishing the two forms by Kulp and Rettger,[9] who also found that most strains of L. acidophilus fermented maltose, sucrose and levulose, whereas most strains of L. bulgaricus did not. L. bulgaricus can also be

34. Davis, J. G., and Rogers, H. J.: J. Hyg. **39**:446, 1939.

35. Orla-Jensen, S.; Otte, N. C., and Snog-Kjaer, A.: Zentralbl. f. Bakt. (Abt. 2) **94**:452, 1936. King and Rettger.[30]

36. Stephenson, M.: Bacterial Metabolism, London, Longmans, Green & Co., 1939, pp. 29 and 55.

37. Sevag, M. G., and Shelburne, M.: J. Gen. Physiol. **26**:1, 1942.

38. Dodds, E. C.: Brit. J. Exper. Path. **5**:183, 1924.

39. Nakai, B.: Shikwa Gakuho **35**:47, 1930.

40. Werkman, C. H.: Bact. Rev. **3**:187, 1939.

41. Snell, E. E.; Strong, F. M., and Peterson, W. H.: J. Bact. **38**:293, 1939.

42. Snell, E. E., and Peterson, W. H.: J. Bact. **39**: 273, 1940.

43. Hill, T. J., and Kneisner, A. H.: J. Dent. Research **21**:467, 1942.

44. Rosebury, T.: Bact. Rev. **8**:189, 1944.

distinguished by its higher optimum temperature for growth. This again, however, is a statistical rather than an absolute distinction. Lazicóvá [45] reported that strains of L. acidophilus isolated from human sources grew best at 37 C. but could be grown at 45 C., whereas strains from industrial sources preferred 45 to 50 C. but could be grown at 37 C. More recently Sherman and Hodge [46] found that L. bulgaricus fails to survive under conditions that permit continued development of L. acidophilus, namely, in a lactose-peptone-yeast broth or in 2.5 per cent sodium chloride or at p_H 7.8. L. bulgaricus seldom grew at 15 C., while L. acidophilus usually did so. Freshly isolated strains of L. bulgaricus grew at 50 C., whereas L. acidophilus did not.

Lactobacilli of the Intestine, the Vagina and the Mouth.—It is generally accepted that the vaginal lactobacilli are indistinguishable from L. acidophilus of the intestine.[47] The relationship of lactobacilli from the mouth and the intestine, on the other hand, has been a point of active controversy. L. acidophilus as defined in the foregoing pages is admittedly heterogeneous; it is possible that the future will disclose valid criteria for its subdivision, and that these will be correlated with the sources of strains. Up to the present, however, only statistical distinctions have been made within the group, and the validity even of these is not beyond question. There is a suggestion in the record, for example, that selected smooth strains from the mouth have been compared with selected rough strains from the intestine, and that such commonly correlated properties as fermentative activity and acidogenic capacity have in consequence been offered as differential criteria. The designation of a bacterial "species" in general is a matter of common and continued difficulty for which thus far no basis has been found universally applicable. Matters of convenience in identification and particularly of differential pathogenicity are the common criteria; whereas, "smoothness" and "roughness" are now accepted as marking variants only, with the implication that either can be converted into the other under appropriate circumstances. The controversy over the relationship of oral to intestinal lactobacilli has been influenced mainly by two considerations: intestinal implantability and the production of dental caries. The imputation of pathogenicity to the oral forms has seemed to be, although actually it is not, in conflict with the therapeutic use of a similar organism. Rettger and his associates [10] have contended that a rough or X lactobacillus should be used for intestinal implantation, and Sullivan, Still and Goldsworthy [16] have argued that lactobacilli which are actively acidogenic in vitro—usually smooth strains—are most likely to be concerned in dental caries. Neither contention has been validated, however, and either would seem, in any event, to be quite inadequate as a basis for the designation of a species. Many workers have found in comparative studies of groups of lactobacilli derived from the mouth, the intestine and the vagina that heterogeneity is the rule and that no consistent difference could be correlated with source of strains. Until more is known on this score the only reasonable course would seem to be the continued designation of all these forms by a single specific name, L. acidophilus.

For those interested in the details of this question it may be stated that Morishita,[48] Curran, Rogers and Whittier,[13] Weinstein, Anderson and Rettger,[49] Ulicny,[50] Gillespie and Rettger [51] and King and Rettger [30] have suggested that oral lactobacilli are distinct from the intestinal ones, while Rosebury, Linton and Buchbinder,[52] Howitt,[53] Hadley, Bunting and Delves,[11] Reploh,[47d] Pesch and Zöllner [54] and Sherman and Hodge [46] have been unable to distinguish the two groups.

Lactobacilli and Streptococci.—The data given in the foregoing pages and in another review (Rosebury [31]) indicate a close relationship between the lactobacilli and the streptococci which has not been generally recognized. For practical purposes the relationship is most apparent when one compares the lactobacilli with the nonpathogenic streptococci, and perhaps it has been overlooked only as a phase of the general neglect that has been visited on both these groups by medical bacteriologists. Among bacteria of medical importance pathogenicity is necessarily the prime criterion for the separation of groups. The manifest pathogenicity of the hemolytic

45. Lazicóvá, T.: Časop. lék. česk. **19**:73, 1939.

46. Sherman, J. M., and Hodge, H. M.: J. Bact. **40**: 11, 1940.

47. (*a*) Heinemann, P. G., and Ecker, E. E.: J. Bact. **1**:435, 1916. (*b*) Jötten, K. W.: Arch. Hyg. **91**: 143, 1922. (*c*) Thomas, S.: J. Infect. Dis. **43**:218, 1928. (*d*) Reploh, H.: Zentralbl. f. Bakt. (Abt. 1) **133**:332, 1935. (*e*) Jesensky, J.: Cesk. stomatol., 1936, p. 285; abstracted, Bull. Inst. Pasteur **35**:505, 1937.

48. Morishita, T.: Proc. Soc. Exper. Biol. & Med. **25**:654, 1928; J. Bact. **18**:181, 1929.

49. Weinstein, L.; Anderson, T. G., and Rettger, L. F.: J. Dent. Research **13**:323, 1933.

50. Ulicny, H. P.: J. Dent. Research **15**:397, 1937.

51. Gillespie, R. W. H., and Rettger, L. F.: J. Bact. **31**:14, 1936; **36**:621, 1938.

52. Rosebury, T.; Linton, R. W., and Buchbinder, L.: J. Bact. **18**:395, 1929.

53. Howitt, B.: J. Infect. Dis. **46**:351, 1930.

54. Pesch, K. L., and Zöllner, A.: Arch. f. Hyg. **116**:295, 1936.

streptococci (and of the related pneumococci) therefore marks them apart. Yet it is a matter of common agreement that these pathogenic forms are closely related to the viridans and lactic streptococci and to the enterococci. The interrelationships of these forms are such as to suggest a continuous spectrum whose bands, despite their own distinctive characteristics, merge insensibly into those on either side. It is not difficult to distinguish a group A strain of hemolytic streptococci from a strain of Str. salivarius; the difficulty enters when one compares a greening variant of the former with a strain of Streptococcus mitis. In like manner it appears that the whole streptococcic spectrum merges with that of the lactobacilli. Typical members of either group are easily distinguished, but intermediate forms exist and are troublesome.

The relationship of lactobacilli and streptococci has been recognized by Bergey and his coworkers,[2] who group the two genera together in the family Lactobacteriaceae, and also by Henrici,[55] whose comment is as follows:

> The Lactobacili parallel the Streptococci in many features. They are found in similar habitats—the mucous membranes (especially the intestines) of animals, on plants, and in milk. They are Gram-positive organisms typically occurring as slender rods in chains. As the Streptococci tend to become elongated, approaching the bacillary form, so the Lactobacilli tend to become shortened, approaching the Streptococcus form; and with a particular strain it is often difficult to decide off hand whether it is a Streptococcus or a Lactobacillus.

The points of similarity between the lactobacilli and the streptococci may be listed as follows:

1. They are similar in surface growth on agar. Colonies of both groups on a medium such as dextrose agar may be indistinguishable. They are usually 1.5 mm. or less in diameter, translucent and finely granular, varying from smooth to rough. Howe and Hatch [23] used the term "streptococcus-like" to describe colonies of lactobacilli.

2. The two groups overlap in cellular appearance. The lactobacilli are typically rod-shaped or filamentous, and the streptococci are typically spherical or oval, but either may take the form of the other. Dawson, Hobby and Olmstead [56] have described bacillary and filamentous forms in rough colonies of both streptococci and pneumococci. The "Streptococcus mutans" of Clarke [57] and MacLean [58] was characterized by the formation of rods in acid mediums; and a strain of greening streptococci with which Hammond and Tunnicliff [59] induced a caries-like process in an extracted tooth, as noted later in this review, grew in the dentin as rods and filaments. Coccal forms have been described frequently in lactobacillus cultures (e. g., Rodriguez,[60] Rosebury, Linton and Buchbinder [52]). McIntosh, James and Lazarus-Barlow [61] noted that lactobacilli from the mouth often resembled streptococci so closely that they could be classified only after repeated subculture. Such streptococcic forms may appear in either smooth or rough colonies of lactobacilli, but are especially noteworthy in certain rough colonies, which may show chains of bizarre organisms, often with cocci and rods in a single chain. It is such forms in particular that may be called lactobacilli by some workers and streptococci by others.

3. They are strikingly similar in metabolism. This would seem to be the most fundamental aspect of their relationship; it is also the one most commonly overlooked. The streptococci and the lactobacilli both lack catalase and cytochromes; both contain flavoproteins and may produce peroxide in the presence of free oxygen, which they use sparingly at most. Members of both groups may produce greening on blood agar, a medium used commonly for streptococci, seldom for lactobacilli. Both groups appear to derive their energy principally or exclusively from the dissimilation of carbohydrates, and both produce lactic acid as the principal product of fermentation. The parasitic members of both groups do not form grossly appreciable amounts of carbon dioxide, but it is of interest that there are gas-forming bacteria related to the streptococci (genus Leuconostoc) and that gas-forming lactobacilli are also known.

It will be seen subsequently that these points of similarity may help to resolve a controversy of long standing in the bacteriology of dental caries. It must be reemphasized, however, that the typical lactobacillus differs clearly from the typical streptococcus: the points given indicate relationship but not identity. Typical strains differ in appearance and in other details that are used for their specific identification. Such typical strains differ, moreover, in three general characteristics:

55. Henrici, A. T.: Biology of Bacteria, Boston, D. C. Heath & Co., 1939, p. 359.

56. Dawson, M. H.; Hobby, G. L., and Olmstead, M.: J. Infect. Dis. **62**:138, 1938.

57. Clarke, J. K.: Brit. J. Exper. Path. **5**:141, 1924.

58. MacLean, A. H.: Brit. Dent. J. **48**:579, 1927.

59. Hammond, C., and Tunnicliff, R.: J. Dent. Research **19**:1, 1940.

60. Rodriguez, F. E.: Mil. Dent. J. **5**:199, 1922.

61. McIntosh, J.; James, W. W., and Lazarus-Barlow, P.: Brit. J. Exper. Path. **5**:175, 1924.

1. The lactobacilli show slower growth in vitro than the streptococci. The latter generally reach their maximum concentration under optimal conditions in twenty-four hours or less, whereas the lactobacilli, even when habituated to suitable mediums, require approximately twice as long. A difference in rate of acid production in culture parallels this difference in rate of growth: the lactobacilli produce acid more slowly.

2. Typical, actively growing (usually smooth) strains of lactobacilli are more strongly acidogenic in vitro than any strain of streptococci. The streptococci do not reduce the p_H of dextrose broth below 4.0, whereas lactobacilli frequently yield levels of p_H 3.5 to 3.8. In terms of total acidity this difference may be as great as fivefold. The lactobacilli, moreover, appear to be more aciduric than the streptococci. It is noteworthy that the difference in acidogenic capacity applies only to selected strains of lactobacilli; many other strains, particularly rough ones, have not been found to induce p_H levels below 4.0.

3. The nonhemolytic streptococci are pathogenic. Members of both viridans and enterococcus groups are responsible for subacute bacterial endocarditis in man; no type of lactobacilli has been clearly implicated in this or any comparable disease (but see the following section). This practical difference is in itself sufficient to warrant the clear distinction made between these two groups.

PARASITIC EFFECTS OF LACTOBACILLI

Pathogenicity.—In routine studies by many workers, lactobacilli have been found incapable of infecting laboratory animals, and there is no reason to incriminate them as etiologic agents in any disease other than dental caries, in which, as will be shown later, their participation need not involve any degree of virulence in the generally accepted sense of that term. There have nevertheless been a few reports of the production of lesions in animals with lactobacilli. Howitt,[62] for example, reported that in rabbits the intravenous injection of large doses was followed by the development of joint lesions with an extensive mucopurulent exudate containing numerous polymorphonuclear leukocytes and macrophages and occasional gram-positive rods. This observation has not been confirmed. Jay, Crowley and Bunting[63] noted the development of sterile abscesses following subcutaneous inoculations of lactobacillus vaccines in both human subjects and rabbits. The production of abscesses seemed to result from the injection of rough but not from that of smooth strains. Similar results were obtained in rats by Rosebury, Foley and Greenberg.[64] These were not transmissible lesions but seemed to be either inflammatory responses to bacterial constituents or allergic phenomena. Canby and Bernier[65] have reported the development of sterile abscesses in rabbits and guinea pigs following inoculations of either living or heat-killed lactobacilli. King and Rettger[30] observed that on intradermal inoculation of rabbits with oral lactobacilli lesions were produced in both sensitized and unsensitized animals. Similar but less severe processes were obtained with killed organisms. Aerobic and anaerobic lactobacilli of intestinal origin did not produce lesions in unsensitized animals.

As a matter of curiosity it may also be noted that in a case of ulcerative (acute) endocarditis Marschall[66] recorded the isolation of L. acidophilus, said to be of the vaginal type, from the blood post mortem. No significance need be attached to this finding, nor to the further one that the patient's serum agglutinated the organism to a dilution of 1:640, since both may be attributed without pathogenic import to the presence of the organism on the mucous membranes.

The lactobacilli, then, have not been successfully indicted as pathogens within the customary meaning of that term. On the contrary, they have been praised for beneficial effects in the vagina and the intestine of the human host. Their relations and effects in these areas and in the mouth may now be considered.

Beneficial Effects in the Vagina.—The evidence for beneficial effects attributable to lactobacilli is particularly striking with reference to the vagina. Here an interesting relationship has been demonstrated between estrogenic function during the period of sexual activity in women, the deposition of glycogen in the vaginal mucous membrane, the proliferation of L. acidophilus in the vaginal secretion and the consequent acid inhibition of other micro-organisms.

Döderlein, in 1892,[67] reported that the vaginal secretion of many pregnant women contains a simple group of micro-organisms, consisting of large numbers of a bacillus, some yeasts and little else. This simple flora, now recognizable as typically aciduric, was associated with a strongly acid secretion. Döderlein found that in

62. Howitt, B.: J. Infect. Dis. **46**:368, 1930.

63. Jay, P.; Crowley, M., and Bunting, R. W.: J. Am. Dent. A. **19**:265, 1932.

64. Rosebury, T.; Foley, G., and Greenberg, S.: J. Dent. Research **14**:231, 1934.

65. Canby, C. P., and Bernier, J. L.: J. Am. Dent. A. **29**:606, 1942.

66. Marschall, F.: Zentralbl. f. Bakt. (Abt. 1) **141**: 153, 1938.

67. Döderlein, A.: Das Scheidensekret und seine Bedeuting für das Puerperalfieber, Leipzig, E. Besold, 1892.

women whose vaginal secretion was less acid, the flora was more varied, and noted that in the latter group there was a higher incidence of puerperal disease. He suggested that the lactic acid produced by the vaginal bacillus limits the growth of other micro-organisms in the vagina and thus protects the area against infection. Many subsequent workers have confirmed and amplified this concept. Menge [68] found that the normal vaginal secretion is sufficiently acid to prevent the growth of streptococci. Hinrichs [69] later reported that the vaginal bacillus grows in vitro at levels below p_H 4.5, whereas other vaginal organisms die off at such levels within a few days. Determinations of the p_H of the vaginal secretions by later workers have been in general accord with these findings, minimal values of p_H 3.9 to 4.0 having been reported by several (Oberst and Plass [70]; Trussell and MacDougal [71]), and it has been generally agreed that a low p_H is associated with a preponderance of Döderlein's bacillus, and vice versa.

Krönig [72] confirmed most of Döderlein's findings and observed in addition that the vagina of the infant has a similar inhibitory power. Döderlein's bacillus, which, as noted, is a member of the acidophilus group, has been found in the vagina at about the third day after birth.[73] Cruickshank and Sharman [74] have since shown that this lactobacillus becomes established in the vagina soon after birth and persists for the first two or three weeks of life, in association with a highly acid, nonpurulent secretion and a simple vaginal flora.[75] After the first month the secretion becomes scanty or absent and when measurable is alkaline; the flora is then normally varied and sparse. At puberty there is a sudden reversion to a simple bacillary flora and a highly acid secretion; these conditions persist in healthy women until the menopause, when the prepubertal sparse varied flora and scanty alkaline secretion return. Soeken [76] had previously suggested that the change from a predominantly coccal to a predominantly bacillary flora in the vagina is the first definite indication of the onset of puberty.

The mechanism underlying these changes in the vaginal flora was indicated by the work of Miura,[77] who postulated a direct association between ovarian activity, deposition of glycogen in the vaginal wall and presence of Döderlein's bacillus and of lactic acid in the vagina. Cruickshank and Sharman [74] observed that the appearance and the disappearance of glycogen in the vaginal wall are closely correlated with changes in the bacterial flora. Glycogen could be demonstrated in the vagina in fetuses, in infants up to the third or fourth week of life and in women during the reproductive period. It was not found during the period from one month after birth to puberty but generally appeared before the onset of menstruation; it was again absent or scanty following either natural or artificial menopause. They suggested that the deposition of glycogen is correlated with ovarian activity, and that the occurrence of glycogen during the first weeks of life depends on maternal estrogen, which appears at this time in the urine of the infant. Cruickshank [78] reported that among the pathogenic or saprophytic bacteria of the vagina examined in vitro only Döderlein's bacillus was able to ferment glycogen, with the production of lactic acid. It is of interest that lactobacilli of intestinal or oral origin were found to ferment glycogen more slowly, or only after adaptation. Glycogen thus appears to serve as a substrate for the production of lactic acid,

> which soon reaches such a concentration that only acid-resistant bacteria such as Döderlein's bacillus can survive and multiply. In this way a defense mechanism is produced in the vaginal cavity capable of preventing the establishment there of foreign and possibly harmful bacteria (Cruickshank and Sharman [74]).

Such a protective mechanism would seem to be most needed during the reproductive period, when the vagina is presumably most subject to exogenous infection. It may also operate during the brief period in childhood when Döderlein's bacillus is present in the vagina. Hardy [79] reported a well marked inverse correlation between the presence of this organism in cultures and the occurrence of pus in smears from the vaginas of infants.

It has since been shown by Beilly [80] that administration of estrogens to castrated women, to women suffering from hypo-ovarianism or to

68. Menge, K.: Deutsche med. Wchnschr. **20**:891, 1894.

69. Hinrichs, K.: Zentralbl. f. Gynäk. **49**:1592, 1925.

70. Oberst, F. W., and Plass, E. D.: Am. J. Obst. & Gynec. **32**:22, 1936.

71. Trussell, R. E., and MacDougal, R. F.: Am. J. Obst. & Gynec. **39**:77, 1940.

72. Krönig: Zentralbl. f. Gynäk. **18**:3; 662, 1894.

73. Schweitzer, B.: Zentralbl. f. Gynäk. **43**:641, 1919.

74. Cruickshank, R., and Sharman, A.: J. Obst. & Gynaec., Brit. Emp. **41**:190 and 208, 1934.

75. These workers and others have nevertheless noted that at birth, before bacteria make their appearance, the vaginal contents are rather strongly acid. G. C. Hardy (Am. J. Dis. Child. **62**:939, 1941) recorded p_H levels below 5 under these conditions and noted that the cells were well filled with glycogen. The origin of this neonatal acidity is not clear.

76. Soeken, G.: Ztschr. f. Kinderh. **40**:727, 1926.

77. Miura, H.: Kyoto-Ikadaigaku-Zasshi **2**:1, 1928.

78. Cruickshank, R.: J. Path. & Bact. **39**:213, 1934.

79. Hardy, G. C.: Am. J. Dis. Child. **62**:939, 1941.

80. Beilly, J. S.: Endocrinology **25**:128, 1939; **26**: 959, 1940.

those in the natural postclimacteric period restores the normal acidity of the secretion, and that the p_H level can thus be used as an objective index both of ovarian function and of the effectiveness of estrogen therapy. Mack and Ale [81] and Mack [82] have reported similar findings for vaginal glycogen. Estrone given orally was found effective in augmenting the glycogen content of vaginal smears, as determined by iodine vapor staining, and such smears were recommended as a test for the therapeutic value of the large variety of natural and synthetic estrogens now available.

Attempts have also been reported at treatment of vaginal infections, such as leukorrhea, by implantation of lactobacilli. The use of L. bulgaricus for this purpose [83] seems irrational, since this organism has been found incapable of continued parasitism. If a strain of lactobacilli is to be used for vaginal implantation it would be best to use a strain isolated from the vagina whose capacity to ferment glycogen has been predetermined. Cruickshank [78] found that L. bulgaricus did not ferment glycogen. Since the data suggest that implantation even of such a strain would be unlikely in the absence of naturally deposited glycogen, which in turn apparently depends on ovarian activity, the validity of lactobacillus therapy of the vagina in the absence of other measures seems doubtful.

Beneficial Effects in the Intestine.—The influence of lactobacilli in the intestinal tract bears only a slight resemblance to that of lactobacilli in the vagina. The problem of the effect on the intestine is much more complex, and its practical implications are correspondingly less clear. Only in the breast-fed infant during the nursing period does the intestinal flora approach in simplicity that of the normal adult vagina. In the bottle-fed baby and in all persons after weaning, lactobacilli are apparently always outclassed by other intestinal micro-organisms.

The development of the intestinal flora from the time of birth was defined by the early studies of Escherich [84] and others, and in particular by those of Tissier.[85] Moro [86] isolated L. acidophilus from the feces of infants and gave it its specific name, but Tissier pointed out in the same year (1900) that this organism is not found normally in the intestine of the breast-fed infant, whereas the related anaerobic form, L. bifidus, which he isolated and named, constitutes the characteristic intestinal flora of such infants during the nursing period. Tissier noted a marked difference in the intestinal flora of breast-fed as compared with bottle-fed infants. He found that the digestive tract is sterile in the first ten to twenty hours after birth but that as the meconium gives way to feces there is a phase of developing infection. In the breast-fed baby during this period, white staphylococci, colon bacilli, spore-bearing anaerobes, enterococci and other bacteria appear in increasing numbers, but they are soon rapidly replaced by a simple flora which persists through the nursing period. L. bifidus makes its appearance toward the end of the mixed phase and proliferates quickly. As it does so, the other bacteria disappear, the large spore-bearing rods first, then the other forms and finally the cocci. This phase of transformation is completed by the third or the fourth day after birth. Thereafter, while the diet remains constant the intestine shows only L. bifidus on direct examination and small numbers of colon bacilli, enterococci and sometimes Aerobacter aerogenes on culture. Other bacteria, including L. acidophilus, according to Tissier, appear only under abnormal conditions in the nursling stool. In bottle-fed infants he found the phase of developing infection more prolonged, its peak being reached toward the fourth day, and he noted no well defined transformation. L. bifidus is present, but colon bacilli, enterococci, L. acidophilus, white staphylococci, sarcinae, A. aerogenes, yeasts and other micro-organisms continue to be found, with none predominant and with a good deal of qualitative variation from infant to infant. He noted that these conditions were not altered when sterilized milk was fed.

Tissier's observations have been generally confirmed by later workers (Snyder [87]). Upton,[88] for example, reported that as long as the meconial character of the stool persists the flora includes a variety of micro-organisms but that most of these disappear under breast feeding, as L. bifidus becomes prominent. L. bifidus may make its appearance in the feces as early as the second day of life and becomes predominant by the fourth or the fifth day in the breast-fed infant, apparently in response to the establishment of the milk diet. In the bottle-fed baby, on the other hand, the varied and variable meconial flora persists, and L. bifidus is found only in small numbers. Different types of artificial feed-

81. Mack, H. C., and Ale, T.: J. Clin. Endocrinol. **2**:361, 1942.

82. Mack, H. C.: Am. J. Obst. & Gynec. **45**:402, 1943.

83. Brady, L., and Reid, R. D.: Ann. Surg. **115**: 840, 1942.

84. Escherich, T.: Die Darmbakterien des Säuglings, Stuttgart, F. Enke, 1886.

85. Tissier, H.: Compt. rend. Soc. de biol. **51**:943, 1899; Ann. Inst. Pasteur **19**:109, 1905; footnote 28.

86. Moro, E.: Wien. klin. Wchnschr. **13**:114, 1900.

87. Snyder, M. L.: J. Infect. Dis. **66**:1, 1940.

88. Upton, M. F.: Am. J. Dis. Child. **37**:1221, 1929.

ing were not found reflected in differences in the intestinal flora.

L. acidophilus seems never to be more than one of many species of micro-organisms that inhabit the intestinal tract. It appears early in bottle-fed infants, later in breast-fed ones, and continues as a common but not a constant part of the intestinal flora in adults. Its presence there has nevertheless given rise to a large amount of literature and to a therapeutic practice that has been widely used. This subject may now be considered.

Intestinal Acidophilus Therapy. — "Change your intestinal flora" was a familiar advertising slogan during the 1920's, one manifestation of a wave of lactobacillus and related intestinal therapy which has since passed its crest. It stemmed particularly from the studies which Metchnikoff [89] undertook during the last years of his life. The subject is of historical interest and has also been curiously interwoven with the problem of the oral lactobacilli and their relationship to dental caries.

Metchnikoff believed that the good health and longevity of many ancient peoples may have depended on their habit of drinking soured milk, such as the Russian koumiss, the Caucasian kefir, the Armenian matzoon and the Balkan yoghurt. These milks were soured by the use of "starters" —particles of the curd of a previously well soured milk, containing lactobacilli of the bulgaricus type, accompanied by lactic streptococci and often also by yeasts. L. bulgaricus having been isolated from milk by Grigoroff in 1905,[90] Metchnikoff suggested that the feeding of milk cultures of this organism might be useful in cases of intestinal putrefaction, with the view that the large amount of acid produced by the lactobacillus would restrain the growth of other bacteria. He hoped as well that he might "postpone and ameliorate old age" by this means. Investigations in support of this thesis conducted by Metchnikoff and his followers (Kopeloff [91]) were encouraging but not convincing, and others failed to support the claims made for L. bulgaricus therapy. The controversy over this question was resolved in favor of the opposition by the studies of Herter and Kendall [92] on a monkey and by those of Rahe [93] with 3 human subjects. These workers reported that L. bulgaricus disappeared from the intestine within a few days after feeding stopped, even when such feeding had been maintained over periods as long as forty-four days. Hull and Rettger [94] and Rettger, Kirkpatrick and Card [95] reported similar experiences with bulgaricus feeding in rats. Although these results left certain doubts unresolved, they seem to have closed the L. bulgaricus chapter of the story of lactobacillus therapy. Thereafter attention was directed to the analogous use of L. acidophilus, which as a natural intestinal parasite offered promise of successful implantation.

The subject of intestinal acidophilus therapy has been reviewed at length by Rettger and Cheplin,[96] Kopeloff [91] and Rettger, Levy, Weinstein and Weiss.[10] In essence these workers have advanced the view that milk soured under suitable conditions with a pure culture of L. acidophilus can be used to implant the lactobacillus in the human intestine and that this use of it is of value in the treatment of chronic constipation and other intestinal disorders. The successful implantation of L. acidophilus apparently results in a simplification of the intestinal flora. Torrey and Kahn [97] have shown that L. acidophilus inhibits the proteolytic activity of Clostridia to a degree closely correlated with the amount of acid produced by it, and Upton [98] found that L. bifidus inhibits the growth of colon bacilli and of enterococci, an effect that seemed to be due more to the production of acetic and formic than to that of lactic acid.

Similar effects have been achieved by feeding lactose or dextrin without acidophilus cultures; the slow absorption of these carbohydrates permits them to reach the lower intestine in sufficient concentration to favor the development of L. acidophilus. To accomplish this purpose, however, objectionably large amounts of these sugars must be fed daily.

Successful therapeutic results with milk cultures of L. acidophilus seem to require painstaking attention in the preparation of the cultures and in their administration. Frequently failure has been attributed to neglect of such details, in particular to a lack of attention to the nature of the organism used. Rettger has been of the opinion that the rough or X type of L.

89. Metchnikoff, E.: Prolongation of Life, New York, G. P. Putnam's Sons, 1908.

90. Grigoroff, S.: Rev. méd. de la Suisse Rom. **25**: 714, 1905.

91. Kopeloff, N.: Lactobacillus Acidophilus, Baltimore, Williams & Wilkins Company, 1926.

92. Herter, C. A., and Kendall, A. I.: J. Biol. Chem. **5**:293, 1908.

93. Rahe, A. H.: J. Infect. Dis. **16**:210, 1915.

94. Hull, T. H., and Rettger, L. F.: Zentralbl. f. Bakt. (Abt. 1) **75**:219, 1914.

95. Rettger, L. F.; Kirkpatrick, W. F., and Card, L. E.: Bulletin 80, Storrs Agricultural Experiment Station, 1915.

96. Rettger, L. F., and Cheplin, H. A.: Transformation of the Intestinal Flora, New Haven, Conn., Yale University Press, 1921.

97. Torrey, J. C., and Kahn, M. C.: J. Infect. Dis. **33**:482, 1923.

98. Upton, M. F.: J. Bact. **17**:315, 1929.

acidophilus is most suitable, but, as previously noted, the superiority of this variety for the purpose does not seem to have been clearly demonstrated. In any event, such difficulties have prevented successful commercial development of this field of therapy, and the lack of a generally available satisfactory preparation, combined with improper commercial exploitation of unsatisfactory preparations, have contributed to a general decline of the practice of acidophilus therapy. I am advised by Kopeloff that he and Rettger continue to practice this therapy successfully. If one may judge from the data given and from the apparent beneficial effect of lactobacilli in the vagina, L. acidophilus therapy would seem theoretically sound, but one need consider only the difficulties incorporated in the large accumulations of varied nutrient materials and in the massive variegated flora of the intestine to underscore the relative complexity of the problem.

Of particular interest from the standpoint of this review is the bearing which these studies of intestinal lactobacillus therapy have had on parallel studies of lactobacilli in relation to dental caries. The implication that a related if not identical lactobacillus, as the causative agent of dental caries, is a "pathogen" has evidently disturbed certain workers in the intestinal field. Students of the oral lactobacilli, for their part, have not been concerned with the need for rigid specifications of a lactobacillus capable of intestinal implantation. The need for such specifications, possibly augmented by a modicum of bias, has led some of those in the opposite camp to suggest that the specific designation L. acidophilus be reserved for implantable forms only, and the attempt has been made to establish the corollary that all other parasitic lactobacilli, but especially the alleged "pathogens" of the mouth, are quite distinct. It may be suggested that a consideration of the data given in this review, undertaken as objectively as my own unavoidable bias will permit, warrants two relevant conclusions: (*a*) that the effort to separate or to delimit bacterial species on grounds such as those mentioned can lead only to confusion and (*b*) that whether or not the lactobacilli are causative agents of dental caries it is misleading to regard them as pathogens. In other words, there is nothing in the record which makes the activity of lactobacilli in the mouth in any way incompatible with an activity in the intestine or the vagina that is beneficial to the host.

Effects in the Mouth.—Bacteria appear in the mouth within a few hours after birth. The flora of the mouth follows a course of development different from that of the flora of either the intestine or the vagina, leading to the establishment of a considerably more varied group of indigenous micro-organisms than is found in either of the other locations. It undergoes no such sharp change as occurs in the flora of the intestine of the nursling with the establishment of breast feeding or in that of the vagina at puberty. Lactobacilli may be members of the first group of micro-organisms to invade the mouth [99]; they seem to appear there a day or two earlier than in the intestine or the vagina [73]; but they do not become predominant at any period and never occur to the exclusion of other micro-organisms. After the establishment of the dentition, the presence and the concentration of L. acidophilus in the mouth are correlated to a significant degree, although by no means absolutely, with the presence of carious lesions of the teeth, as will be noted later. It has been suggested that their correlation with carious activity, a quantity thus far insusceptible of direct measurement, is even more marked. That the presence of lactobacilli in the mouth is related in a physical manner to the presence of tooth surfaces has been indicated by the finding of Bradel and Blayney [100] that lactobacilli can seldom be found in edentulous mouths but that they reappear in a high percentage of instances within two weeks after the placement of artificial dentures. The relationship of lactobacilli to dental caries is discussed in detail in the following sections.

LACTOBACILLI AS A CAUSE OF DENTAL CARIES: PRO

W. D. Miller,[101] building on the earlier studies of Leber and Rottenstein [102] and Underwood and Milles,[103] first clearly formulated the so-called chemicoparasitic theory, which attributes dental caries to bacterial action at localized areas of the tooth surface where particles of food tend to collect and persist. Caries of enamel, according to this view, is due directly to dissolution of that tissue by the acids of bacterial fermentation,

99. Brailovsky-Lounkevitch, Z. A.: Ann. Inst. Pasteur **29**:379, 1915.

100. Bradel, S. F., and Blayney, J. R.: J. Am. Dent. A. **27**:1601, 1940.

101. Miller, W. D.: (*a*) Arch. f. exper. Path. u. Pharmakol. **16**:291, 1882; (*b*) Micro-Organisms of the Human Mouth, Philadelphia, S. S. White Dental Manufacturing Co., 1890.

102. Leber, T., and Rottenstein, J. B.: Untersuchungen über Caries der Zähne, Berlin, A. Hirschwald, 1867; cited by Miller.[101b] Dental Caries and Its Causes, and Introduction into the Influence of Fungi in the Destruction of the Teeth, London, J. & A. Churchill, 1878.

103. Underwood, A. S., and Milles, W. T.: Tr. Internat. M. Cong. **3**:523, 1881.

while caries of the underlying dentin is regarded as due to this process and, in addition, bacterial digestion or proteolysis of the matrix of the dentin. Miller found that the altered appearance of natural carious enamel could be duplicated roughly by treating intact enamel with dilute acids in vitro, that fluid expressed from washed fragments of carious dentin was acid to litmus, that dentin softened by caries had undergone marked loss of lime salts and that the widened tubules of carious dentin and its interglobular spaces and "liquefaction foci" were filled with masses of micro-organisms. He found, moreover, that pieces of sound tooth which had been kept in a mixture of saliva and bread in vitro at 37 C., with frequent changes of the medium over a three month period, underwent changes in both enamel and dentin that were indistinguishable from those of natural caries. Miller's bacteriologic studies, however, were imperfect. He had little success in the cultivation of bacteria from carious teeth. He believed caries of both enamel and dentin to be bacteriologically nonspecific, largely because he observed in stained sections that carious tubules may contain cocci, bacilli or filaments. These different forms were frequently disposed so that a single tubule was occupied by only one morphologic type. This observation has been confirmed by subsequent work, but it will be seen that it is not incompatible with the view of a more specific bacteriologic causation. Miller's emphasis on caries of dentin, moreover, conditioned by his inability to deal effectively with caries of enamel, led him to base his conclusions on late phenomena rather than on initial changes in the disease. Subsequent work has nevertheless confirmed the general soundness of his views in all respects except those concerned with details of the bacteriologic nature of dental caries. Adequate treatment of this subject was possible only by use of the pure culture methods of later workers.

It was not until 1915 that the first adequate methods for this purpose were developed. Earlier studies had tended either to confirm Miller's idea of nonspecific infection or to emphasize the streptococci because of their prominence in simple cultures. Goadby [104] seems to have been the first to record the association of lactobacilli with dental caries, but he attached no special importance to them, merely listing them under the name Bacillus necrodentialis along with streptococci and staphylococci as among the acid-forming bacteria in the deep layers of carious dentin.

104. Goadby, K. W.: The Mycology of the Mouth, London, Longmans, Green & Co., 1903.

Kantorowicz [105] found the same three groups of bacteria in this location but reported that the streptococci far outnumbered the others, and he considered them most important. Niedergesäss [106] observed that bacilli predominated in a varied flora in the superficial layers of early caries of dentin but that in the deeper layers cocci increased relatively and that in the deepest layers only staphylococci and streptococci could be found. It is of interest that similar observations were reported much more recently by Harrison [107] in studies of the bacteria of experimental lesions in the teeth of rats.

For the ultimate prevention of dental caries a knowledge of the nature of its onset would seem more important than an understanding of its later course. Caries of enamel and caries of dentin are probably not the same, and since enamel is nearly always the first tissue to be attacked, the emphasis in the foregoing studies on an attempt to determine the vanguard flora in dentin may have been misplaced. For this and other reasons, particular significance may be attached to the work of Kligler [108] (1915). It was he who, as a result of careful quantitative studies of the bacterial changes that accompany the onset of caries of enamel, first proposed the lactobacilli as the most important of the bacteria concerned in the initiation of the carious lesions. He found that the numbers of bacteria per milligram of surface deposit on the teeth were from ten to fifty times larger in mouths with early caries than in caries-free mouths, as determined both directly in microscopic counts and on aerobic and anaerobic plates. On tooth surfaces in caries-free mouths 44 per cent of the strains isolated were streptococci, over 70 per cent were cocci of all types, only 2 to 5 per cent were non-spore-bearing bacilli and 15 per cent were filamentous organisms. Similar results were obtained with specimens from healthy tooth surfaces in "clean" mouths with a history of caries, and values only about twice as high were obtained with specimens from noncarious surfaces in "dirty" mouths. From carious surfaces, on the other hand, the percentage of cocci of all kinds dropped to 40 or 50, that of streptococci alone to 20, while the thread forms and "gram-positive acidific bacilli" increased respectively to about 28 and 25 per cent. Kligler identified the filamentous forms as Cladothrix placoides and Leptothrix buccalis. These organisms are discussed further else-

105. Kantorowicz, A.: Bakteriologische und histologische Studien über die Caries des Dentines, Leipzig, G. Thieme, 1911.

106. Niedergesäss, K.: Arch. f. Hyg. **84**:221, 1915.

107. Harrison, R. W.: J. Infect. Dis. **67**:97, 1940.

108. Kligler, I. J.: J. Allied Dent. Soc. **10**:141, 282 and 445, 1915.

where.[44] The former is now difficult to identify, but it may have been a rough lactobacillus or an actinomycete, while the latter appears to have been a common oral saprophyte of no established significance. Kligler attributed to these organisms the function of attaching a bacterial mass firmly to the tooth surface by virtue of their growth as a filamentous network. His acid-producing bacilli were clearly identified as L. acidophilus. Regarded as identical with Goadby's B. necrodentalis, they were found capable of elaborating and withstanding a greater amount of acid than any of the other types and were emphasized as the important agents in inducing the primary decalcification of enamel in caries.

These findings did not satisfy workers who regarded the streptococci as of particular importance in dental caries. Hartzell and Henrici,[109] for example, pointed out that Kligler's data indicate an absolute increase of streptococci in caries despite the relative decrease. It may also be noted that even in the maximal flora of incipient caries, according to Kligler, the streptococci are still prominent relatively as well as absolutely. Additional evidence for the importance of lactobacilli was soon developed, however, by other means.

The profusion of varieties of micro-organisms in the mouth and the opportunities for contamination with extraneous forms have been a source of continued difficulty. Among the means devised to overcome this difficulty have been those which attempt deliberately to select certain varieties on the basis of a working hypothesis which attributes special importance to them. Thus Howe and Hatch [28] postulated that the more essential bacteria of dental caries should be best adapted to the environment of a carious tooth and therefore best able to survive in it over prolonged intervals. Accordingly, they placed fillings over open cavities and left them in position for six weeks to three months in an attempt to kill off the extraneous flora. When the fillings were removed aseptically and the underlying carious dentin was cultured, 10 of 27 teeth showed no growth, and the other 17 yielded only lactobacilli, including both L. acidophilus and L. bifidus. Similar results were obtained after fillings with slight antiseptic properties had been placed for shorter periods.

A related hypothesis has become the foundation of nearly all later work on the role of lactobacilli in dental caries. It was first applied by McIntosh, James and Lazarus-Barlow,[110] who stated their thesis in the following terms:

> . . . Miller considered that the production of acid by bacteria was the initial process [in dental caries]. We therefore have endeavored to discover a bacterium or group of bacteria present in dental caries which can produce sufficient acid to decalcify enamel and dentine. Once decalcification has been produced, the path is left open for almost any microorganism to invade the dentine.
>
> It is reasonable to suppose that the bacteria which produce large quantities of acid will be able to live in relatively high concentrations of acid. Therefore, bacteria which are able to decalcify enamel must be able to live in a very acid medium.

These workers were accordingly the first to use a medium of low initial p_H for the cultivation of bacteria from carious cavities. By this means they were able to observe lactobacilli in cultures of material from 48 of 50 teeth and to isolate them in 44, or 88 per cent. Other organisms were isolated only irregularly. They named the organisms Bacillus acidophilus odontolyticus, and classified them into two types, chiefly on morphologic grounds. Unfortunately the p_H measurements of these workers, performed with the hydrogen electrode, seem to have been subject to a considerable error, as Rosebury [15] has pointed out. They obtained best results with a broth medium whose p_H was given as 3.5, and they reported that with dextrose broth the end point p_H values ranged from 3.4 to 2.2, the average being given as 2.75. These values can be brought into line with those of other workers by assuming a constant error of — 1.3 to — 1.5 p_H, which would correct the p_H values for broth to p_H 4.8 or 5.0 and the lowest end point p_H to 3.5 or 3.7.

In the same year Rodriguez,[60] in parallel but independent studies, also isolated lactobacilli from clinically noncarious dentin under deep cavities, called them Bacillus odontolyticus and attempted to classify them into three types by morphologic differences. He likewise found final p_H values for dextrose broth (2.9 to 3.9) which were significantly lower than those of other workers and were probably erroneous. Confirmatory results were also reported by Sierakowski and Zajdel.[111]

In their first report McIntosh and his collaborators [110] stated that extracted sound teeth which had been placed in dextrose broth cultures of a lactobacillus and transferred aseptically to fresh dextrose broth every eight days showed after several months a picture closely similar microscopically to caries of dentin except that the organisms penetrated the dentin from the pulp side. It seems noteworthy that this picture included typical foci of liquefaction, involving loss of organic substance in the dentin, despite

109. Hartzell, T. B., and Henrici, A. T.: J. Nat. Dent. A. **4**:477, 1917.

110. McIntosh, J.; James, W. W., and Lazarus-Barlow, P.: Brit. J. Exper. Path. **3**:138, 1922.

111. Sierakowski, S., and Zajdel, R.: Compt. rend. Soc. de biol. **91**:961, 1924.

the fact that none of the lactobacillus cultures was able to liquefy gelatin or produce indol in vitro—properties universally absent among the lactobacilli. Similar results, moreover, could not be obtained when Str. salivarius or Escherichia coli was substituted for a lactobacillus. These organisms produced no macroscopic changes in five and two and one-half months, respectively, while Staph. aureus, inoculated with artificially decalcified dentin, produced proteolysis much more marked than that of natural foci of liquefaction. In a later report, McIntosh, James and Lazarus-Barlow [61] stated that when whole teeth were covered with celluloid varnish except for a small area of enamel and then placed in dextrose broth inoculated with a lactobacillus as before,

> . . . the picture of "natural" caries was reproduced in every respect; the organisms destroyed the enamel, decalcified part of the dentine and passed down the dentinal tubules for a short distance.

Under similar circumstances Str. salivarius produced decalcification without apparent dentinal infection and without foci of liquefaction. This picture of artificial caries could not be duplicated by Bunting and Palmerlee,[112] who obtained only decalcification in extracted teeth covered with shellac except for a window and exposed to lactobacilli, and they noted that the lesion was histologically unlike that of natural caries.

The association of lactobacilli with dental caries and their ability to decalcify teeth, if not to produce caries, have been abundantly confirmed since 1925, particularly by the work of Bunting and his collaborators. Abandoning the use of extracted teeth, these workers returned to in vivo methods to obtain material for bacteriologic studies. Their findings may be grouped under the following heads:

Qualitative Correlation of Lactobacilli with Dental Caries.—By the use of dextrose-containing mediums, p_H about 5, cultures made of material from tooth surfaces or of saliva yielded lactobacilli in 77 to 99 per cent of various groups with caries and failed to yield lactobacilli in 66 to 100 per cent of various groups without caries, in a total of over 1,000 cases.[113] As these authors pointed out, but for a difficulty in making a precise diagnosis of active caries—in view of the circumstance, that is, that cavities in some mouths may be nonprogressive and therefore bacteriologically atypical—the percentages in the caries groups might have been higher. Technical errors, moreover, would be on the side of negative cultures. Such considerations would presumably not apply to caries-free mouths, some of which are thus found to contain lactobacilli. These findings have been confirmed by Thompson,[114] Enright, Friesell and Trescher [115] and others.

Quantitative Correlation of Lactobacilli with Dental Caries.—Rodriguez [116] devised a method for the quantitative plating of lactobacilli in saliva and reported that the numbers isolated were a presumptive index of caries activity. His method was complicated, involving the use of serum agar incubated anaerobically with 10 per cent carbon dioxide, and probably for this reason it was not adopted by other workers. On the other hand, the simpler aerobic quantitative method of Hadley,[4] utilizing streaked tomato agar, has been generally accepted and widely used. By this method, Jay, Hadley, Bunting and Koehne [117] found that repeated cultures of the saliva, taken with paraffin stimulation in such a way as to include abundant debris from the tooth surfaces, yielded consistently high concentrations of lactobacilli from persons with dental caries and either negative cultures or inconsistent low counts from persons free from dental caries. In the former group the counts are found to range from about 50,000 to about 700,000 lactobacilli per cubic centimeter of saliva, whereas in the caries-free group the count seldom exceeds 10,000 per cubic centimeter. These findings have been confirmed by Rosebury and Waugh,[118] who showed that with the Hadley method the only micro-organisms isolated whose occurrence is correlated with dental caries are lactobacilli and yeasts, the former in a higher degree, and that only the lactobacilli are correlated quantitatively with caries. Similar results have been reported by Snyder.[119] Jay [120] and others have applied the Hadley method widely as a diagnostic test for caries activity.

Appearance of Lactobacilli Before the Clinical Onset of Caries.—On making repeated cultures

112. Bunting, R. W., and Palmerlee, F.: J. Am. Dent. A. **12**:381, 1925.

113. (*a*) Bunting, R. W.; Nickerson, G., and Hard, D. G.: Dent. Cosmos **68**:931, 1926. (*b*) Bunting, R. W.; Nickerson, G.; Hard, D. G., and Crowley, M.: ibid. **70**:1, 1928. (*c*) Bunting and Palmerlee.[112]

114. Thompson, R.: Proc. Soc. Exper. Biol. & Med. **29**:103, 1931.

115. Enright, J. J.; Friesell, H. E., and Trescher, M. O.: J. Dent. Research **12**:759, 1932.

116. Rodriguez, F. E.: J. Am. Dent. A. **17**:1711, 1930; **18**:2118, 1931.

117. Jay, P.; Hadley, F. P.; Bunting, R. W., and Koehne, M.: J. Am. Dent. A. **23**:846, 1936.

118. Rosebury, T., and Waugh, L. M.: Am. J. Dis. Child. **57**:871, 1939.

119. Snyder, M. L.: (*a*) J. Dent. Research **18**:497, 1939; (*b*) J. Am. Dent. A. **29**:2001, 1942.

120. Jay, P., in Gordon, S. M.: Dental Science and Dental Art, Philadelphia, Lea & Febiger, 1938, pp. 348-372.

over a period of about six months of material from the mouths of children free from caries, Jay and Voorhees [3] noted that in certain children whose mouths consistently yielded lactobacilli dental caries subsequently developed. Bunting, Nickerson, Hard and Crowley [118b] have confirmed this observation. More recently Blayney, Bradel, Harrison and Hemmens [121] reported somewhat similar results on repeated cultivation of scrapings from individual proximal surfaces of selected teeth in children. During the period of study 56 of these areas became carious, as determined by periodic clinical and roentgenologic examinations. In this group the percentage of areas from which lactobacilli could be isolated rose from 20 to 38 at four and a half months before clinical onset and, after remaining at this level during two additional examinations at six week intervals, rose to 43 per cent at the time of onset and then continued to rise slowly, to a maximum of 77 per cent nine months after onset. These experiments were not quantitative, and the low percentages may have been influenced by the difficulty of obtaining an adequate inoculum from a single intact tooth surface.

An additional point which supports the view that lactobacilli are etiologically related to dental caries is the following:

Acidogenic and Aciduric Capacity of Lactobacilli.—Evidence has been presented in this and preceding sections which suggests that lactobacilli produce and withstand higher concentrations of acid than other bacteria of mucous membranes. The final p_H values of standard dextrose infusion broths in which lactobacilli are actively growing characteristically range between 3.5 and 3.9. The streptococci, which seem to approach the lactobacilli as closely in this respect as any parasitic group, yield end point p_H values under similar conditions of 4.0 or higher. It seems plain, moreover, that lactobacilli can survive under conditions which other bacteria find inimical to continued growth (e. g., in the vagina, as already noted). Rosebury [15] reported that certain strains of lactobacilli could reduce the p_H of a weakly buffered medium containing dextrose toward a limiting value of about 3.0. Under similar conditions Staph. aureus and E. coli were found to yield p_H values approaching the limit 4.0. The general significance of this is obvious. It may have an important relation to the solubility of enamel at various p_H levels, as noted by Karshan and Rosebury.[122]

Snyder and Teachout [123] have studied the acidogenic and aciduric properties of 30 strains each of lactobacilli, streptococci, staphylococci and yeasts, all isolated from the mouth. The streptococci included both alpha and gamma strains, while the staphylococci were chiefly of the albus type. Only smooth strains of lactobacilli, which, as noted, are usually more acidogenic in vitro than rough strains, were included. Of 30 strains of lactobacilli, all grew on tomato agar at p_H 4.7, and 25 grew at p_H 4.5, the lowest level tested. Of the staphylococci, all 30 strains grew at p_H 5.0, 25 at p_H 4.7 and 10 at p_H 4.5. Twenty-eight of 30 strains of streptococci grew at p_H 5.5, but only 2 grew at p_H 5.3 and none at p_H 5.0 or lower. In addition, all 30 strains each of lactobacilli, yeasts and staphylococci were found to survive for ten days in dextrose broth and to yield average final p_H values of 3.8, 5.0 and 4.7, respectively. Five of 30 strains of streptococci, on the other hand, were nonviable after twenty-four hours in dextrose broth, and only 2 strains survived in this medium for ten days. The average final p_H of the medium in which these organisms grew was 4.6. Except for the yeasts, whose ability to survive in an acid environment (but not to produce acid) is probably at least as great as that of the other forms, the lactobacilli were found to be both most acidogenic and most aciduric.

Divergent results have been reported by Florestano,[124] who stated that a group of streptococci gave average final p_H values as low as 3.6, compared with 3.8 for smooth lactobacilli and 3.9 for staphylococci, all strains having been isolated from the mouth on tomato agar. This worker's results are also in disagreement with those of most others in that he was able to isolate streptococci and staphylococci from the mouth with regularity on tomato agar of p_H 5. Rosebury and Waugh [118] could isolate streptococci from the mouths of only 2 of 106 Eskimos by the use of this medium, and staphylococci from the mouths of 22. Both strains of streptococci and 17 of the strains of staphylococci were from mouths with no clinically detectable active caries. Snyder [119a] also reported that streptococci could be recovered from saliva only irregularly by the Hadley method and somewhat more often from mouths without than from those with caries. He isolated staphylococci more frequently but likewise irregularly.

121. Blayney, J. R.; Bradel, S. F.; Harrison, R. W., and Hemmens, E. S.: J. Am. Dent. A. **29**:1645, 1942.

122. Karshan, M., and Rosebury, T.: J. Dent. Research **14**:220, 1934.

123. Snyder, M. L., and Teachout, J. J.: J. Dent. Research **21**:461, 1942.

124. Florestano, H. J.: J. Dent. Research **21**:263, 1942.

LACTOBACILLI AS A CAUSE OF CARIES: CONTRA

In the foregoing section the results of many groups of investigators who, working independently, have reached substantial agreement on the data are reviewed. It is in recognition of human fallibility, of the elusiveness of truth and the ubiquity and seductiveness of error that the method of independent confirmation has achieved an established place in science. The four points enumerated in the foregoing section, which bear directly on the association of lactobacilli with dental caries, have all been independently confirmed. However, the report of Florestano discussed in the last paragraph of that section demonstrates that confirmation need not imply unanimity. Others have also reported results at variance with those just given. One group of such results, dealing with the role of streptococci in dental caries, will be considered in a separate section later. The others seem to contradict those of the preceding workers. They exemplify the controversial nature of research on dental caries, which in turn reflects the many-sided complexity of the problem. It may be noted that the positive findings in the bacteriologic examination of dental caries seem to imply a causation in which only factors in the immediate environment of the teeth are concerned. In other fields of research, notably that of diet and nutrition, data have developed that seem to imply a systemic causation of dental caries. It is a curious fact that several of the reports to be considered in this section represent attempts by members of the nutritionist school to check the findings previously discussed. It happens that there is no essential conflict between these two camps; there is truth on both sides. Conflict has its origin, at least in part, in an attempt to oversimplify the problem, which is evidently neither all black nor all white but an intermediate gray. One way to resolve this conflict is to inquire carefully into the basis of the divergent results. The answer can be obtained or at least surmised in most instances from a close examination of the reports in question.

Tucker [125] stated that he was unable to correlate the incidence of dental caries in a group of 422 children with any one type of organism grown from tooth scrapings in broth of p_H 5. Streptococci were isolated from nearly all the mouths irrespective of the presence or the absence of caries. Lactobacilli were isolated most frequently and most consistently from the mouths of children with three or more cavities, but they were also obtained from mouths without caries or with caries arrested for two years. It was concluded that "L. acidophilus is not an obligate producer of dental caries." The divergence here seems to be one of interpretation only. Previous data have not suggested that the occurrence of lactobacilli is correlated with the number of carious lesions in the mouth, a quantity which obviously need bear no relation to the activity of caries in that mouth. Karshan, Rosebury and Waugh [126] were also unable to correlate either the presence of lactobacilli or their concentration as shown by Hadley counts with the percentage of carious teeth, although the correlation with caries activity as determined clinically was unmistakable. Other workers are agreed, moreover, that lactobacilli can be recovered from caries-free mouths.

Boyd, Zentmire and Drain [127] were also impressed with their ability to recover lactobacilli from the mouths of children without caries or with caries arrested by dietary treatment. Their chief finding, however, was based on a method described earlier by Magee, Drain and Boyd.[128] Dextrose broth, p_H 5.7, inoculated with saliva was tested for p_H after twenty-four hours' incubation in an effort to determine the "acid-producing capacity of the oral flora." The p_H values ranged between 4.0 and 4.8, with those for broth containing L. acidophilus no lower than those for broth containing only other micro-organisms. The divergence here seems to be a technical one. The data of Snyder and Teachout,[123] previously mentioned, make it clear that the difference in inhibitory power between a medium of p_H 5.7 and one of p_H 5.0 is by no means insignificant. The higher level used by Boyd and his collaborators would be expected to permit a considerably wider range of growth. A twenty-four hour incubation period, furthermore, would not permit maximum growth of lactobacilli, which usually require at least forty-eight hours. This study can therefore not be offered seriously as justifying the authors' statement that

> . . . any effect attributable to bacteria in the destruction of tooth substance must be considered secondary in nature, acting upon a tooth whose defense against decay has been depressed through sub-optimal conditions operating from within the organism.

The authors were evidently not fully convinced themselves, since several years later they returned to the question,[129] this time to check the

125. Tucker, W. H.: J. Infect. Dis. **51**:444, 1932.

126. Karshan, M.; Rosebury, T., and Waugh, L. M.: Am. J. Dis. Child. **57**:1026, 1939.

127. Boyd, J. D.; Zentmire, Z., and Drain, C. L.: J. Dent. Research **13**:443, 1933.

128. Magee, C.; Drain, C. L., and Boyd, J. D.: Proc. Soc. Exper. Biol. & Med. **26**:718, 1929.

129. Speidel, T. D.; Boyd, J. D., and Drain, C. L.: J. Dent. Research **18**:185, 1939.

findings of others by the Hadley quantitative method. They studied a group of 65 diabetic children under dietary management, 26 of whom were reported to have "some degree of active caries," while the other 39 had "inactive or no caries." Determinations of the p_H of primary dextrose broth cultures were again made, but this time the medium had an initial p_H of 5.0 and the cultures were incubated for fifty and one hundred hours. The results obtained were not presented in full. A chart indicated that little or no correlation was found between the terminal p_H values of these broth cultures and the concentrations of lactobacilli obtained on tomato agar plates: values as low as p_H 3.7 were shown, and one value of p_H 3.8 seems to have been obtained in the absence of lactobacilli. This report may perhaps be looked on with skepticism in view of the data given previously. The other results of these workers were presented in a negative and seemingly overcomplicated manner: Lactobacilli were not found in 17 per cent of 122 determinations on the 26 children with caries, nor in 40 per cent of 65 determinations on the 36 children with arrested or no caries. It is stated that "in general the highest incidence of Bacillus acidophilus was found in cultures from children with active caries"; yet the authors concluded that "neither incidence of B. acidophilus in large quantities nor high acid producing capacity showed marked correlation with activity or inactivity of dental caries." When one considers that the subjects of this study were all diabetic children under dietary control, presumably on low carbohydrate diets which may have depressed the numbers of salivary lactobacilli (the highest count recorded in the chart was 110,000 per cubic centimeter), these findings do not seem to be in serious conflict with those given previously.

A final paper in this series, the contents of which are more difficult to reconcile with those of other papers, is that of Anderson and Rettger.[130] These workers were unable in any instance to isolate lactobacilli from the saliva of persons with caries on a tomato agar of p_H 5.0 similar to that of Hadley. Streptococci were isolated from most of the subjects under these conditions. When scrapings or "decay material" from extracted teeth was used as the inoculum in place of saliva, 6 of the 33 subjects had lactobacilli on the p_H 5.0 medium, 12 streptococci and 7 yeasts. All of these subjects except 1 were listed as having caries of some degree, arrested in 3, presumably active in the remainder. These results seem to be in unavoidable conflict with those of other workers, and no explanation of the conflict is evident in the report. It is noted that the lactobacilli isolated were of the smooth type, and the suggestion is made that they are distinct from (intestinal) L. acidophilus. Rettger's views on this subject have been discussed in an earlier section. It is conceivable that some organisms which other workers would have classed as lactobacilli were called streptococci by these workers, but such an explanation would account for only part of the discrepancy. It seems of passing interest that for a number of years my students have used the Hadley method as a class exercise, plating their own saliva, and that each year a majority of them have had no difficulty in recognizing the typical colonies of lactobacilli, both smooth and rough, and in finding the characteristic gram-positive rods in their microscopic preparations.

130. Anderson, T. G., and Rettger, L. F.: J. Dent. Research **16**:489, 1937.

STREPTOCOCCI AS A CAUSE OF DENTAL CARIES

Several reports dealing with streptococci in caries have already been mentioned. It is noted that before 1922, when media selective for lactobacilli were first applied to this problem, the prominence of streptococci in the mouth led such workers as Kantorowicz,[105] Niedergesäss,[106] and Hartzell and Henrici [109] to attach considerable importance to them as agents of dental caries. Most workers in this field have since abandoned this view, but others continue to adhere to it. Clarke, for example, reported in 1924 [57] that streptococci described as a distinct species, Streptococcus mutans, were isolated in acid mediums from 36 of 50 carious teeth, whereas lactobacilli were obtained from only 14, 11 of which also yielded streptococci. The strain was described as producing cocci in chains in neutral or alkaline mediums and bacilli in acid mediums. On isolation it did not grow below p_H 5.6. It produced acid rapidly and yielded p_H 4.2 in twenty-four hours, but did not exceed this level. Cross agglutination tests suggested that it was distinct from any type of lactobacilli and also from Str. salivarius, Str. faecalis and from hemolytic streptococci. It was found capable of decalcifying teeth and was said to be more effective than lactobacilli in doing so. MacLean [58] later reported that Str. mutans could be distinguished from L. acidophilus in that the former was less aciduric, had more constant fermentation reactions and grew in smaller, darker colonies. He also reported that there was no cross agglutination between the two organisms with low titered serums. McIntosh, James and Lazarus-Barlow,[131] on the other hand,

131. McIntosh, J.; James, W. W., and Lazarus-Barlow, P.: Brit. J. Exper. Path. **6**:260, 1925.

were unable to recover Str. mutans from carious lesions "with any degree of constancy," and cultures of the organism given to them "produced only a faint degree of acidity and typical caries lesions were not observed." Later workers have likewise failed to duplicate the findings of Clarke and MacLean. Tefft [132] has reported without details that large numbers of Str. faecalis and Streptococcus lactis as well as Str. salivarius, could be found in caries. The identity of Str. mutans has remained uncertain.

Bibby, Volker and Van Kesteren [133] have reported data which they interpreted as supporting the view that streptococci rather than lactobacilli are important agents of dental caries. These workers rejected the hypothesis, adopted by most others, that the essential micro-organisms of caries may be selected by virtue of their aciduric properties, arguing that the numbers of a given kind of micro-organism and the rate of growth and of acid production may be more significant. They isolated lactobacilli from caries on tomato agar and selected a group of "acidogenic" bacteria, on dextrose-ascitic fluid Douglas agar containing bromcresol purple indicator, as the group that changed the indicator completely, representing a p_H of about 5.2 or lower. By this criterion they determined that lactobacilli made up less than one tenth of the acidogenic flora of the mouth and that streptococci were much more abundant. Since streptococci, as judged by titrations at intervals during growth, produced acid more rapidly than lactobacilli, they argued that at p_H ranges in the mouth streptococci would produce acid in greater quantity:

> . . . since the streptococci are approximately 100 times more numerous than the lactobacilli and since it is likely that activity in the mouth parallels that in test-tubes, it can be estimated that lactobacilli give rise to no more than .025 per cent of the acid formed by the action of salivary organisms on carbohydrates.

It has been noted previously that the assumption of parallelism between bacterial activity in the test tube and that in the mouth is gratuitous and doubtful. The lag of growth in artificial cultures, the uncertain nutritive optimum and the imperfect disposal of metabolic products which retard bacterial growth in the test tube are probably not duplicated in vivo, where the integrated growth may have no lag at all and where the other conditions may be more perfectly adapted to continued proliferation. Conversely, certain conditions antagonistic to bacterial growth in the mouth are not duplicated in the test tube. Nearly all heterotrophic bacteria, whether parasitic or saprophytic, moreover, are "acidogenic"—acid production by dissimilation of carbohydrates is a nearly universal energy-yielding mechanism of bacteria as well as of animal and plant cells. The data of Bibby and his co-workers indicate that lactobacilli, although they grow and produce acid more slowly in vitro than streptococci, eventually yield considerably greater quantities of acid than any of the other bacteria tested. Their data are also in harmony with the observations of others in that they show a close relationship between the concentration of lactobacilli in saliva and the presence of caries and indicate that the average number of streptococci found was considerably higher in noncarious than in carious mouths. Their findings are therefore in agreement with those of workers who favor the lactobacilli as agents of caries.

The data which more than any others seem to incriminate as agents of caries certain streptococci are those that point to the capacity of the latter to produce in vitro a dental lesion remarkably similar microscopically to the picture of bacterial invasion of dentin in natural caries. It has been noted that McIntosh, James and Lazarus-Barlow [134] reported the production of such lesions with lactobacilli and stated that they were unable to produce them with streptococci, including Str. mutans. Their results [121] were illustrated with drawings showing a bacillary invasion of the dentinal tubules with the formation of apparently typical foci of liquefaction. It has also been noted that Bunting and Palmerlee [112] could not duplicate this result, and that the implication of proteolytic activity of lactobacilli is not in accord with laboratory findings. Tunnicliff and Hammond [135] reported that a rough greening streptococcus isolated from pulp and dentin of carious teeth grew in bacillary forms, often palisading and producing crescentic and coiled individuals, straight or undulating filaments or chains of bacilli with occasional coccal forms. These rough colonies could be transformed into smooth ones by transferring through dextrose broth every two or three days for from three to twelve transfers; after about three months a stabilized culture of smooth colonies was obtained which showed only cocci in pairs and short chains. Hammond and Tunnicliff [59] were then able to produce artificial caries in extracted teeth with this smooth variant and observed in sections that the dentinal tubules contained bacilli, coiled forms and filaments, as well as cocci, in a manner closely resembling that observed in natural caries. These observations were illustrated with convincing photomi-

132. Tefft, H. I.: J. Dent. Research **21**:318, 1942.

133. Bibby, B. G.; Volker, J. F., and Van Kesteren, M.: J. Dent. Research **21**:61, 1942.

134. Footnotes 61 and 110.

135. Tunnicliff, R., and Hammond, C.: J. Am. Dent. A. **25**:1046, 1938.

crographs. The data given seem adequate to establish the organism as a greening streptococcus but are unfortunately insufficient to identify it more fully. The possibility that it may have been a rough lactobacillus or a form intermediate between the two genera can be decided only after confirmation and extension of the observations. It was noted that the organism was not strongly acidogenic (lowest final p_H, 4.4), that it was unencapsulated, insoluble in bile, greening on blood and avirulent for mice and young rabbits. A careful comparative study of lactobacilli and streptococci isolated from dental caries, particularly of rough forms of both genera, to determine the possible relationship or identity of intermediate forms, might illuminate this problem and help to resolve the whole lactobacillus-streptococcus controversy in this field.

ROLE OF YEASTS IN DENTAL CARIES

Yeasts are the only micro-organisms other than lactobacilli whose occurrence on selective mediums like tomato agar of p_H 5 has been found correlated with the presence or the absence of dental caries. Yeasts appear on such plates as large cream-colored or buff-colored smooth dull opaque colonies and appear in Gram stains as large round, oval or elongated cells in which the characteristic budding is often conspicuous. They have been found in about one third of the carious mouths examined, less often in non-carious mouths. Unlike the lactobacilli, they usually appear in low concentration, and in mouths that yield them they have not been found quantitatively related to dental caries.[118] The association of yeasts with dental caries was apparently first noticed by Howe and Hatch [23] and has also been reported by Bunting and Palmerlee [112] and by Snyder.[119a] Knighton [136] has presented a detailed report on the yeasts of the mouth, including a review of the literature. Using Sabouraud's agar and acid dextrose broth, he did not find yeasts correlated with dental caries. Monilia albicans was the species most frequently isolated. It was found pathogenic for rabbits but was not associated in Knighton's subjects with thrush or other disease of the mouth. Other species of Monilia and members of the general Cryptococcus and Saccharomyces were also recovered.

It has already been noted that the yeasts of the mouth may be fully as aciduric as the lactobacilli but that they are much less active in the production of acid. A role in dental caries has been suggested for them particularly by Fosdick and his collaborators.[137] These workers postulated a symbiotic relationship between yeasts and lactobacilli in caries, in which the former micro-organisms, because of their more rapid production of the fermentation intermediate, pyruvic acid, enable the lactobacilli to produce lactic acid more rapidly in combination than in pure culture. The bacteriologic data offered in support of this interesting concept have unfortunately not been convincing. The lactobacilli studied appear to have been poor growers and therefore poor acid formers in artificial mediums. Actively growing lactobacilli are known to be capable of producing higher concentrations of acid than Fosdick and Hansen were able to produce with lactobacillus-yeast mixtures. Snyder and Teachout [123] compared the ability of mixtures of lactobacilli with yeasts, staphylococci or streptococci to acidify bromcresol green dextrose agar with that of lactobacilli and the other species in pure culture and noted no evidence that the mixtures were more effective than lactobacilli alone. This question seems worth further study, but in view of the inconstant appearance of yeasts in mouths with caries it seems unlikely that these micro-organisms can be indispensable in the disease.

IMMUNOLOGIC ASPECTS OF DENTAL CARIES

Immunity in the familiar sense of increased resistance to recurrence of a disease is unknown in dental caries. Enamel and dentin do not heal; once lost in caries they are not regenerated. The disease may be arrested by removal and prosthetic replacement of carious tissue or may undergo apparently spontaneous arrest without operative treatment. Spontaneous arrest may be accompanied in dentin by increased resistance to further progress of the disease in that area. The dentin in such an arrested carious lesion may appear sclerotic and acquire a polished surface. The nature of this change is not well understood. The data of MacPhee [138] suggest that an antigen-antibody reaction may be concerned in it. Such a mechanism, like the deposition of new "secondary" dentin at the expense of pulp, would operate to retard or arrest the lesion but would have no influence on the inception of the disease and would accordingly have no direct bearing on the question of the cause of dental caries. The data to be reviewed in this section deal with certain immunologic phenomena relating to lactobacilli in dental caries.

136. Knighton, H. T.: J. Dent. Research **18**:258, 1939.

137. Fosdick, L. S., and Hansen, H. L.: J. Am. Dent. A. **23**:401, 1936. Fosdick, L. S.; Hansen, H. L., and Wessinger, G. D.: ibid. **24**:1445, 1937. Hansen, H. L.; Fosdick, L. S., and Epple, C. F.: ibid. **24**:1611, 1937.

138. MacPhee, G. G.: Brit. Dent. J. **57**:308 and 351, 1934.

Studies of immunologic factors in dental caries have been based largely on the assumption that such factors may prevent the development of lactobacilli in the caries-free mouth. It seems probable that these bacteria are repeatedly introduced into all mouths. Nearly all workers are agreed that they can be recovered, although irregularly and in small numbers, from caries-free mouths. Rosebury and Waugh [118] found lactobacilli in small numbers in the mouths of caries-free Eskimos in an area of Alaska in which caries was almost unknown. Yet it also seems clear that lactobacilli do not proliferate in caries-free mouths as they do in the presence of caries. Bunting, Crowley, Hard and Keller [139] observed that continual feeding of milk cultures containing large numbers of mouth lactobacilli did not result in the implantation of these organisms in the mouths of caries-free persons who had previously yielded negative cultures. In short, lactobacilli repeatedly introduced by natural means into all mouths seem in some to take up residence and proliferate, and these mouths are usually found to have dental caries; in others they maintain no more than a starveling existence, and these mouths are usually found not to have caries. If lactobacilli are the active agents of dental caries, this circumstance is of crucial importance: What is it that favors the development of lactobacilli in certain mouths? What prevents it in others? Many answers have been offered. This review is concerned only with that one of them which postulates the activity of immunologic factors tending to inhibit the growth of lactobacilli in the caries-free mouth.

Antibodies to lactobacilli have been found in saliva and in blood; cutaneous reactions have been demonstrated by inoculation of lactobacilli, and immunization against dental caries has been attempted both in animals and in man by the use of lactobacillus vaccines. In general it has been found that the occurrence of circulating antibodies is associated with freedom from caries or with low oral lactobacillus counts; conversely, skin reactivity has been observed chiefly in persons with caries, whose mouths usually harbor large numbers of lactobacilli. It has been reported that vaccination with lactobacilli reduced human oral lactobacillus counts; but this treatment has not yet been shown to influence the prevalence of dental caries.

Antibodies to lactobacilli have been found in saliva only irregularly and in low titer, and no special significance has been attached to them in this medium.[140] Johnston and her coworkers [141] and Jay [120] were unable to find agglutinins to lactobacilli in saliva.

Serum agglutinins to lactobacilli have been reported to occur regularly in caries-free persons, to a maximum titer of 1:640, whereas the serum of persons with caries yielded agglutinins irregularly, the highest titer being 1:80 (Jay and associates [142]). Fischer [143] is also said to have demonstrated agglutinins in the serum of 3 caries-free persons in titers from 1:160 to 1:320. The more recent observations of Dietz, Williams and Lawton [144] are not in full agreement with those just cited, although they tend in the same direction. These workers compared the serum agglutinins for 12 strains of lactobacilli with Hadley counts of saliva in 15 cases of caries and 15 of freedom from caries. They detected agglutinins in both groups to a maximum titer of 1:320, but in most instances the titer did not exceed 1:80. Their data show no consistent relationship between presence and titer of agglutinins, on one hand, and either lactobacillus count or carious status on the other. They did note, however, that the highest serum agglutinin titers generally accompanied zero Hadley counts, while high Hadley counts were associated with low serum titers. On the other hand, low serum titers accompanied low or zero lactobacillus counts in several subjects. Their positive findings are thus in accord with those of Jay and his collaborators.

Jay, Crowley and Bunting [63] have reported that filtrates of pooled cultures of lactobacilli produced skin reactions of the delayed type on intradermal inoculation of human subjects with caries. Similar reactions were obtained less frequently in caries-free persons. An attempt at immunizing human subjects by subcutaneous inoculation of a polyvalent lactobacillus vaccine was abandoned because of severe reactions. Experimentally in rabbits it was found that rough strains produce sterile abscesses while smooth strains are innocuous. Smooth lactobacilli, however, although clearly antigenic in rabbits, failed to produce serum agglutinins in human sub-

139. Bunting, R. W.; Crowley, M.; Hard, D. G., and Keller, M.: Dent. Cosmos **70**:1002, 1928.

140. Rosebury, T.: J. Dent. Research **10**:403, 1930. McIntosh and others.[131]

141. Johnston, M. M.; Williams, C. H. M.; Anderson, P. G.; Drake, T. G. H.; Tisdall, F. F., and Kaake, M. J.: J. Am. Dent. A. **23**:1493, 1936.

142. Jay, P.; Crowley, M., and Bunting, R. W.: J. Dent. Research **12**:429, 1932. Jay, P.; Crowley, M.; Hadley, F. P., and Bunting, R. W.: J. Am. Dent. A. **20**:2130, 1933.

143. Fischer, T. E., cited by Dietz, V. H.; Williams, N. B., and Lawton, W. E.: J. Am. Dent. A. **30**:838, 1943.

144. Dietz, V. H.; Williams, N. B., and Lawton, W. E.: J. Am. Dent. A. **30**:838, 1943.

jects.[120] Rosebury, Foley and Greenberg [64] have reported that small groups of rats fed caries-producing diets and immunized by repeated intraperitoneal injections of living lactobacilli had no less caries than nonimmunized animals and that lactobacilli persisted in the mouths of all the animals. Jay [120] also has been unable to prevent caries in rats by immunization with lactobacilli.

Canby and Bernier [65] have continued the studies of Jay. They found that in rabbits and guinea pigs smooth lactobacilli seemed to be more easily phagocytosed than rough strains but that all produced sterile abscesses in varying degree when inoculated subcutaneously. King and Rettger,[30] as noted previously, reported the production of local lesions in rabbits following intradermal inoculation of smooth oral lactobacilli. Canby and Bernier stated that they were able to overcome these toxic effects by dilution. Using heat-killed and phenolized lactobacillus vaccines, standardized by preliminary tests in guinea pigs, they vaccinated 20 human subjects, all with caries, by intradermal inoculation every three to five days. In later inoculations they gave as many as four injections simultaneously to a single subject, each of 0.1 cc. Each subject received from nine to forty-nine injections over a period of about ten weeks, with no severe reactions. In general the agglutinin titer of the serum was increased by this treatment, although not markedly or consistently. The lactobacillus count of saliva was found to fall in all but 1 instance. In this exceptional subject no agglutinins could be demonstrated in the serum either before or after vaccination. The report provided no data on the response to vaccination in terms of dental caries.

This reported reduction in the oral lactobacillus count after vaccination is a striking finding. It calls for confirmation and needs to be correlated with a parallel change in the activity of dental caries as determined by clinical criteria —the only completely satisfactory test for the effectiveness of such method. Except for this finding none of the data presented in the foregoing paragraph necessarily implies any convincing relationship with dental caries. The frequent presence of a micro-organism on a mucous membrane may in itself lead to the development of circulating antibodies or cutaneous reactions to its contained antigens, none of which need interfere seriously with the continued parasitism of the micro-organism. This has been found true of greening streptococci [145] and of Treponema microdentium,[146] among other species. It seems reasonable to assume, moreover, that differences in the concentration of a parasite of mucous membranes like the lactobacillus may be correlated with differences in antibody response to it without any noteworthy influence on the persistence or the activity of the parasite. Over long periods, for example, the high concentration of lactobacilli found in the mouths of persons with caries might stimulate and then absorb circulating antibody and might stimulate skin-reactive antibody without absorbing it. In the caries-free person, with lactobacilli present in the mouth only occasionally and in small numbers, the antigenic stimulus may be quantitatively sufficient to provide the low serum titers obtained but not to absorb the antibody formed. Or the phenomenon of cutaneous sensitivity may be due to an antigen in lactobacilli different from that which induces the formation of circulating antibody. This skin-sensitizing antigen in the caries-free person may be introduced parenterally in dosage insufficient to result in demonstrable sensitivity.[147]

Jay [120] has himself remarked that "it is difficult to understand . . . how circulating antibodies would influence the activity of lactobacilli on the surface of enamel." One may go further, indeed, and suggest that the peculiar characteristics of dental caries are such as to make it seem highly unlikely that either circulating or fixed tissue antibodies can play an important part in it. Until data are available which establish an immunologic basis for dental caries, therefore—or which show that the disease can be prevented by vaccination—a skeptical attitude toward the question seems indicated.

ROLE OF INFECTION IN DENTAL CARIES

The data that have been reviewed seem to establish the indispensability of micro-organisms in the production of dental caries and point toward the lactobacillus as the principal if not the exclusive agent in initiating the actual breakdown of the tooth. It is clear, on the other hand, that the mere presence of such a micro-organism in the mouth need not induce the disease. Something else seems to be required—the presence or the absence of something which either enables or prevents the proliferation of the lactobacillus in the mouth and which in con-

145. Howell, K. M., and Corrigan, M.: J. Infect. Dis. **42**:149, 1928. Nye, R. N., and Seegal, D.: J. Exper. Med. **49**:539, 1929.

146. Kolmer, J. A.; Kast, C. C., and Lynch, E. R.: Am. J. Syph., Gonor. & Ven. Dis. **25**:300, 1941.

147. This view is suggested by the data of W. B. Sherman, A. Stull and R. A. Cooke (J. Allergy **11**:225, 1940) and of M. H. Loveless (J. Immunol. **47**:165, 1943) on the response to pollen desensitization in human subjects.

sequence permits or inhibits its destructive effects.

This point may be emphasized by certain observations on rats, in which an experimental disease closely similar to dental caries in man can be produced by dietary means.[148] Lactobacilli are found in the rat's mouth and can be recovered from it whether caries is present or not.[149] The quantitative data of Harrison[150] indicate that the relationship of lactobacilli to lesions of the teeth in rats is similar to that in man but less marked in degree—their relative increase during the period of development of the lesions is less pronounced. The production of dental caries in rats does not require the deliberate inoculation of lactobacilli or other micro-organisms but is accomplished by the institution of appropriate dietary measures alone. In the absence of such measures the feeding of micro-organisms does not result in the development of caries,[151] and in the presence of the caries-producing dietary conditions the further addition of lactobacilli has not been found to alter the carious response of the animals.[152]

These experimental findings and others, as well as the results of studies of the natural disease in man, are all compatible with the view that the micro-organisms of dental caries are indigenous parasites of the mouth which act to produce dental caries only by taking advantage of other conditions. These other conditions have generally been investigated without direct reference to their effects on the micro-organisms. In 2 instances, however, the effects on the oral lactobacilli of conditions known to influence dental caries have been recorded. These are (1) the reduction of dietary carbohydrate[153] and (2) the presence in drinking water of traces of fluorine.[154] These two measures may perhaps be considered only as exemplifying a more general phenomenon. Both exert a mitigating effect on the prevalence or the activity of dental caries, and this effect is accompanied by a diminution of the oral lactobacillus count.

Is dental caries, then, an infectious disease? The question cannot be answered categorically. There is no evidence that caries is either communicable or transmissible, but if one accepts the evidence that micro-organisms play an indispensable role in the disease, to what degree can their role in it be termed infection? Dental caries appears to be one of several disease processes in which indigenous parasites of the mouth, ordinarily harmless, participate as pathogens. But dental caries is peculiar in that the tissues in which its manifestations appear—excepting cementum, which is affected only uncommonly—do not contain cells and are incapable either of inflammatory response to injury or of healing or regeneration. Correlatively, dental caries is peculiar in that the bacteria which the bulk of the evidence incriminates as exerting the immediate causative action—the lactobacilli—seem altogether lacking in both the aggressive and the defensive attributes customarily included within the meaning of the word "pathogenicity." They seem to produce the disease merely by growing on the tooth surface and producing acids by fermentation of carbohydrate substrates—in other words, merely by following a habit of life common to nearly all micro-organisms (as well as other cells) but following it with peculiar tenacity and potency. When the chemistry of infections comes to be better understood than it is at present, dental caries may seem to differ only in degree—in the simplicity of its pathogenesis—from more typical infections. At present the word "infection" can be applied to this process only if it is postulated that dental caries lies at one end of a range which reaches at the other extreme to infections of superlative virulence such as that of Bacillus anthracis in cattle or that of Pasteurella pestis in man. In anthrax and plague virulence is maximal and resistance minimal, and minute doses of the causative agent may result in overwhelming infection. In dental caries this relationship appears to be completely reversed: Virulence is vanishingly small or absent entirely, while factors in the environment of the micro-organism assume nearly or quite exclusive importance and in themselves determine the event of disease. As a corollary of this argument, the word "resistance" would be applicable to these phenomena only by extension of its customary meaning.

148. Rosebury, T., in Gordon, S. M.: Dental Science and Dental Art, Philadelphia, Lea & Febiger, 1938, pp. 299-318.

149. Rosebury, T., and Karshan, M.: J. Dent. Research **11**:121, 1931.

150. Harrison, R. W.: J. Infect. Dis. **67**:106, 1940.

151. Lilly, C. A.: J. Nutrition **5**:175, 1932. Etchells, J. L., and Devereux, E. D.: Proc. Soc. Exper. Biol. & Med. **30**:1042, 1933. Rosebury, T., and Foley, G.: J. Dent. Research **14**:359, 1934. Rosebury.[148]

152. Rosebury, T.; Karshan, M., and Foley, G.: J. Dent. Research **14**:231, 1934.

153. Howitt, B. F., and Fleming, W. C.: J. Dent. Research **10**:33, 1930. Koehne, M.; Bunting, R. W., and Morrell, E.: Am. J. Dis. Child. **48**:6, 1934.

154. Dean, H. T.; Jay, P.; Arnold, F. A.; McClure, F. J., and Elvove, E.: Pub. Health Rep. **54**:862, 1939.

SUMMARY

The parasitic lactobacilli of the mouth, the intestine and the vagina of the human being

are grouped under the names Lactobacillus acidophilus (the aerobic forms) and Lactobacillius bifidus (the anaerobic forms). The aerobic lactobacilli form a heterogeneous group, but attempts to subdivide them, based on variations in form of colony, on type of lactic acid produced and on growth capacity and acidogenic potency in vitro have generally yielded only statistical distinctions of little practical value. Recent data suggest that a satisfactory classification may be arrived at by serologic means. Two types of L. bifidus have been distinguished, one of which is persistently anaerobic and appears distinct, while the other is anaerobic only on primary isolation and seems otherwise to be closely related to L. acidophilus. The lactobacilli resemble the streptococci in their relation to oxygen and in their energy-yielding mechanisms. The properties of L. acidophilus may intergrade with those of the saprophytic L. bulgaricus. The intestinal and the vaginal lactobacilli are admittedly alike, while the oral and the intestinal forms, although they have been actively compared as a point of controversy, have not been clearly differentiated. The interrelationship of the lactobacilli and the streptococci suggest that an ambiguous intermediate group may exist.

The parasitic lactobacilli appear to be essentially nonpathogenic, despite scattered reports of lesions produced with pure cultures. They seem to play a distinctly beneficial role in the human vagina, where glycogen, deposited as a result of estrogenic function, serves as a substrate for their proliferation and the resultant high acidity inhibits the development of other micro-organisms. Anaerobic lactobacilli predominate in the intestinal flora of nursing infants but are subsequently outnumbered by other forms. Attempts have been made to promote the predominance of lactobacilli in the intestine by feeding L. bulgaricus and, later, L. acidophilus, but the practice of "acidophilus therapy" has diminished in recent years, apparently in large measure because of technical difficulties. Lactobacilli appear in the mouth early in life and persist in relation to dental caries and in apparent dependence on the presence of tooth surfaces.

There is much evidence to implicate lactobacilli in the causation of dental caries. They have been observed to increase markedly in numbers during the onset of caries of enamel. When mediums of low p_H are used, on the hypothesis that the micro-organisms of caries should be resistant to acid, lactobacilli are recovered from carious lesions with regularity. They have been found capable of decalcifying enamel and dentin. They have been recovered in a high percentage of instances from tooth surfaces or saliva of persons with dental caries, and in only a small percentage from caries-free persons. They have been found present consistently and in high concentration in mouths with caries and inconsistently and in low concentration in caries-free mouths. They have been reported to occur in the mouth regularly, or to increase in numbers, before the clinical onset of caries. Finally, lactobacilli seem to be the most actively acidogenic of the oral micro-organisms.

Several reports are at variance with those that incriminate lactobacilli as causative agents of caries. In most instances either the disagreement depends on interpretation rather than on the data presented or the divergences can be attributed to differences in technic. Similar criticisms may be made with respect to several reports in which streptococci rather than lactobacilli have been advanced as the bacterial cause of dental caries. Nevertheless it is noted that the artificial caries in extracted teeth most closely approximating the natural picture of bacterial invasion of dentin has been produced with a rough greening streptococcus. A study of the ambiguous lactobacillus-streptococcus intermediates mentioned in a foregoing paragraph may throw light on this question.

As to the possible role of yeasts in dental caries, the evidence that is available indicates that they are probably not essential agents of the disease.

The immunologic data on circulating antibodies and on skin reactivity to lactobacilli, both of which have been found correlated with the presence of caries, may have no direct connection with the disease but may depend rather on the difference in concentration of lactobacilli in the mouths of persons with and without caries and hence on the intensity of the antigenic stimulus which they provide. Recent data, however, suggest that the number of lactobacilli in the mouth may be reduced by the administration of killed lactobacillus vaccines, although no effect on dental caries itself has yet been demonstrated.

Although the role of micro-organisms in dental caries appears to be indispensable, the part played by infection may be surpassed in the etiology of the disease by other factors which serve to encourage or to restrain the inciting microbial activity. Dental caries can be spoken of as an "infectious disease" only if it is recognized as an extreme example in which parasitic factors —those of "virulence"—are minimal while factors acting in the environment of the parasite are maximal.

Book Reviews

Trichinosis. By Sylvester E. Gould, M.D., D.Sc., pathologist and director of laboratories, Eloise Hospital, Eloise, Mich.; assistant professor of pathology, Wayne University College of Medicine, Detroit. Pp. 356, with 128 illustrations. Price $5. Springfield, Ill.: Charles C Thomas, Publisher, 1944.

The book opens with an interesting historical account of trichinosis, illustrated with portraits of outstanding contributors to the present knowledge of the disease. The life cycle and morphologic aspects of Trichinella spiralis are described and illustrated fully. Then come chapters on the epidemiology of trichinosis, on the disease as it occurs in animals, on its pathologic and immunologic features, including eosinophilia, on diagnostic methods, on the symptom complex and on the diagnosis, the treatment, the prognosis and the control. The text may be "heavy with bibliography" but it presents concisely well documented digests of the literature. The illustrations are uniformly adequate and instructive. The bibliography is extensive if not exhaustive. It is arranged according to years of publication from 1822 to and including at least part of 1943, and is supplemented by an author index. The number of publications in recent years reveals the increasing recognition of the importance of trichinosis. The subject index appears to be detailed and helpful. The monograph tells well the complete story of trichinosis and will be of great value to all who are actively concerned with the disease in man or in animals.

AN OUTLINE OF TROPICAL MEDICINE. By Otto Saphir, M.D., Director of the Department of Pathology of the Michael Reese Hospital; Professor of Pathology, University of Illinois College of Medicine. Price, $1. Pp. 86. Chicago: The Michael Reese Research Foundation, 1944.

The purpose of this small book is to help physicians in the recognition of tropical diseases in warriors returning from tropical regions "as a ready reference and as a bridge to the larger and more comprehensive works on the subject." It does summarize well essential information regarding important tropical infections.

Notes and News

The Scientific Exhibit, American Medical Association, Philadelphia.—Application blanks for space in the Scientific Exhibit at the Session of the American Medical Association to be held in Philadelphia June 18 to 22, 1945 are now available. The period for applications will close on Feb. 12, 1945. The representative to the Scientific Exhibit from the Section on Pathology and Physiology is Dr. F. W. Konzelmann, Atlantic City Hospital, Atlantic City, N. J.

Requests for application blanks may be addressed to him or to The Director, The Scientific Exhibit, American Medical Association, 535 N. Dearborn Street, Chicago 10, Ill.

INDEX TO VOLUME 38

Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in alphabetical order under the letters B and O, respectively.

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CONTENTS